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DIAGNOSIS AND TREATMENT OF CHRONIC COCCIDIOIDOMYCOSIS

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The recognition of acute coccidioidal infection occurring in more or less epidemic form, particularly during the dry, dusty months in endemic areas, may offer little difficulty.[1] However, the bizarre nature of chronic coccidioidal infection may often make the diagnosis of this form of the disease more elusive. With the military movement of troops, soldiers trained in areas in which coccidioidomycosis is endemic may exhibit the manifestations of chronic coccidioidal infection in localities far removed from the site in which the infection was originally acquired and in regions where this diagnosis is rarely considered. It is therefore highly important that medical officers, even though they are stationed well outside of areas in which coccidioidomycosis is endemic, be familiar with the chronic or residual manifestations of this disease. The military implications of coccidioidomycosis have been appreciated by the Surgeon General of the United States Army. In 1941 the preventive medicine division of the medical corps established an extensive program of investigation as part of the program of the Commission on Epidemiological Survey, of the Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army. In collaboration, the Army Air Forces Western Flying Training Command has an extensive program for the control of coccidioidomycosis in operation and has prepared a comprehensive brochure on this subject.[2] This brochure and other recent articles[3] contain extensive bibliographies, and it is not our purpose in this primarily clinical article to duplicate the reviews of the current literature.

We have been particularly interested in the chronic phase of primary coccidioidomycosis and in disseminated coccidioidal infection inasmuch as the patients we have had an opportunity to observe have come under our care weeks to months after the illness first manifested itself. The basis for this report consists of observation of 14 patients proved to have clinical coccidioidomycosis and of 30 patients for whom the diagnosis was proposed but not confirmed. Of the 14 patients for whom the diagnosis was proved, 10 had primary benign and 4 had progressive disseminated coccidioidomycosis. The patients with disseminated infection were included in the series because the addition of the data relative to their conditions helps to stress some of the diagnostic features that we wished to emphasize. The data concerning these 14 patients are noted in table 1. These patients were observed in various station hospitals before being transferred to Hammond General Hospital at Modesto, Calif. The length of time elapsing between the onset of disease and the entry to this hospital for these 14 patients ranged from four weeks to fourteen months and averaged over nineteen weeks (table 2). The data are striking when it is appreciated that the pulmonary infiltrate in acute primary coccidioidomycosis often disappears in about five or six weeks. That the diagnosis of sporadic coccidioidomycosis may be difficult is shown by the fact that 8 of the 14 patients were transferred to Hammond General Hospital with erroneous diagnoses (table 3).

In addition to the 14 proved cases of the disease, the data for 30 additional patients in whom

1. (*a*) Dickson, E. C.: Coccidioidomycosis: The Preliminary Acute Infection with Fungus Coccidioides, J. A. M. A. **111**:1362-1364 (Oct. 8) 1938. (*b*) Faber, H. K.; Smith, C. E., and Dickson, E. C.: Acute Coccidioidomycosis with Erythema Nodosum in Children, J. Pediat. **15**:163-171 (Aug.) 1939. (*c*) Davis, B. L., Jr.; Smith, R. T., and Smith, C. E.: An Epidemic of Coccidioidal Infection (Coccidioidomycosis), J. A. M. A. **118**:1182-1186 (April 4) 1942. (*d*) Goldstein, D. M., and Louie, S.: Primary Pulmonary Coccidioidomycosis, War Med. **4**:299-317 (Sept.) 1943.

2. Coccidioidomycosis Control Program for the Army Air Forces Western Flying Training Command, Santa Ana, Calif., Office of the Surgeon General, Sept. 15, 1943.

3. (*a*) Smith, C. E.: Coccidioidomycosis, M. Clin. North America **27**:790-807 (May) 1943. (*b*) Baker, E. E.; Mrak, E. M., and Smith, C. E.: The Morphology, Taxonomy and Distribution of Coccidioides Immitis Rixford and Gilchrist 1896, Farlowia **1**:199-244 (July) 1943.

TABLE 1.—*Data for Fourteen Patients with Coccidioidomycosis*

Patient No.	Age, Yr.	Race	Onset	Roentgenographic Data	Leukocytes: Number per Cu. Mm. of Blood	Leukocytes: % of Polymorphonuclear Leukocytes	Erythrocyte Sedimentation Rate	Duration of Fever	Cutaneous Reaction to Tuberculin	Cutaneous Reaction to Coccidioidin	Serologic Reaction to Coccidioidin	Coccidioides Demonstrated	Comment
1	32	Filipino	Acute pneumonic	Density in lower lobe of left lung near hilus; multiple cavities	10,200	61	Rapid for 6 months	4 months (temperature 99 to 99.4 F.)	Positive	Positive	Positive	Yes	Slow clearing of densities; patient became asymptomatic and returned to duty 8 months after onset of illness
2	25	White	Acute pneumonic	Consolidation in lower lobe of left lung; cavity appeared 7 months after onset	20,000	74	Rapid for 10 months	5 months (temperature 99 to 99.4 F.)	Negative	Positive	Positive	Yes	Cavity unchanged for 5 months; phrenicolysis performed; cavity disappeared 3 weeks later; patient returned to duty 13 months after onset of illness
3	28	White	Insidious cough; loss of weight	Area of patchy infiltration in upper lobe of left lung; cavity discovered 1 year later	9,400	62	Normal since discovery of cavity	No fever since cavity was discovered	Negative	Positive	Negative	No	Negative reaction to coccidioidin before onset of illness; cavity persisted for 9 months though decreased in size; phrenicolysis and shot bag therapy; patient returned to duty
4	28	White	Unknown; infection accidentally discovered	Solitary nodular density in lower lobe of left lung	8,200	65	Normal	No fever since entry	Negative	Positive	Negative	No	Patient asymptomatic throughout course; negative reaction 9 months before onset of illness; patient returned to duty
5	44	White	Insidious cough; pain in chest	Infiltration in upper lobe of left lung followed by cavitation	7,200	74	Normal after cavity was discovered	2 weeks	Negative	Positive	Negative	No	Attempt to fill cavity with iodized poppyseed oil unsuccessful; patient asymptomatic throughout course after cavity was discovered; discharged from service
6	52	White	Acute pneumonic	Infiltration in lower lobe of right lung with fibrosis	14,800	80	Normal after 1 month	3 weeks	Positive	Positive	Negative	Yes	History of asthma for 4 years; bronchogram showed bronchiectasis; patient retired from service
7	26	White	Unknown; infection accidentally discovered	Cavity in upper lobe of left lung	10,400	66	Normal	No fever since entry	Positive	Positive	Positive	Yes	Negative cutaneous reaction to coccidioidin 2 months previous to onset of illness; shot bag therapy used; cavity disappeared 8 months after it was discovered; patient returned to duty
8	31	White	Unknown; infection accidentally discovered	Cavity in upper lobe of right lung	5,900	59	Rapid for 6 months; now drooping	No fever since entry	Negative	Positive	Negative	No	Negative reactions to 3 cutaneous tests with coccidioidin before onset of illness; cavity smaller though still present 7 months after discovery
9	28	White	Acute pneumonic	Density in lower lobe of right lung, replaced by triangular shadow suggestive of atelectasis	15,900	68	Rapid; now falling 6 months after onset	5 weeks	Negative	Positive	Positive	Yes	Patient asymptomatic 2 months after onset of illness; bronchoscopy revealed no obstruction to bronchus; triangular shadow persisted
10	29	White	Insidious cough	Fibrous strand-like infiltration in upper lobe of left lung	10,600	62	Normal	None	Negative	Positive	Positive	Yes	Patient has had hemoptysis; bronchoscopy shows no abnormality; patient asymptomatic at time of report
11	26	Mexican	Acute pneumonic with pleural effusion	Consolidation followed by effusion	16,150	74	Rapid; dropping at time of transfer	7 months	Positive	Positive	Positive	Yes	Abscess over mediastinum incised; titer of antibodies, temperature and sedimentation rate dropping when patient discharged to veterans' hospital because of dementia precox
12	31	Mexican	Acute pneumonic with pleural effusion	Consolidation followed by pleural effusion	13,200	76	Rapid (8 months after onset)	5 months (temperature 99 to 99.6 F.)	Positive	Positive	Positive	Yes	Backache developed 8 months after onset of illness; roentgen examination showed destructive lesion of tenth thoracic vertebra; titer of antibodies and sedimentation rate decreased.
13	31	White	Insidious weakness; fever; pain in chest	Distinct widening of superior mediastinum	12,700 (10% eosinophils)	81	29 mm. in 1 hour	6 weeks	Positive	Positive, becoming negative	Positive	Yes	Prominent enlargement of supraclavicular glands on the right side; cutaneous lesion on left side of forehead; septic fever treated with penicillin; patient improving at time of report
14	25	White	Insidious cough; pain in chest; headache and weakness	Infiltration at hilus of right lung; cleared in 2 months	15,850	75	Extremely rapid for 2 months; decreasing	4 weeks	Negative	Positive	Positive	Yes	Granulomatous cutaneous lesions developed early in disease; C. immitis identified from one of these lesions

the presence of coccidioidal infection was suspected but subsequently not confirmed, have been analyzed. Fifteen of these patients were admitted to this hospital with a diagnosis of coccidioidomycosis, based mainly on the presence of a positive cutaneous reaction with coccidioidin, without other confirmatory data. This phase of the problem is discussed in the section on the cutaneous test with coccidioidin. Although the number of patients observed is relatively small, they were carefully studied, and from the studies we have assessed the relative clinical value of certain diagnostic aids. Likewise we have learned of diagnostic pitfalls a knowledge of which may benefit others who care for patients with this disease.

TABLE 2.—*Length of Time Elapsing Between Onset of Disease and Entry to Hammond General Hospital in Fourteen Cases of Coccidioidomycosis*

Time	Number of Cases
4 weeks	2
6 weeks	1
8 weeks	1
11 weeks	1
3 months	3
4 months	2
5 months	1
9 months	2
14 months	1

TABLE 3.—*Transfer Diagnoses for Fourteen Patients with Coccidioidomycosis*

	Number of Patients
Coccidioidomycosis	6
Pleurisy with effusion	2
Tuberculosis, pulmonary	1
Pneumonia, unresolved	1
Pneumonia, type undetermined	1
Pneumonia, primary atypical	1
Pneumonia, with atelectasis	1
Pulmonary abscess	1

DIAGNOSIS

Aids in diagnosis of chronic coccidioidal infection may be grouped conveniently as follows: (1) history, (2) cutaneous test with coccidioidin, (3) cultures and inoculation of animals, (4) serologic tests with coccidioidin, (5) studies of tissues, (6) studies of the blood, particularly of the sedimentation rate, and (7) roentgenologic studies.

History.—A history of exposure in an area in which the disease is endemic, particularly during the warm, dry, dusty months, is of extreme importance as a clue to the diagnosis. The endemic areas in the United States are the southern half of California, particularly the San Joaquin Valley, Southern Arizona and Western Texas. Other endemic areas may exist in the United States, but there is not sufficient evidence at the present time to permit definite conclusions concerning them. The Chaco region of Argentina, from which the first case of coccidioidomycosis was reported in 1892,[4] is undoubtedly an endemic area. It is also possible that the disease may exist in other dry, arid, dusty regions of the world.

All of our patients gave a history of being stationed at a post in an endemic area. Most of them underwent routine military training in these areas; several had been stationed in more than one endemic locality, and 3 contracted the disease while on maneuvers in highly infected terrain. The exposure of 1 patient was more difficult to ascertain.

A 31 year old Mexican (patient 12, table 1), formerly a resident of Chicago, was a patient in a station hospital in an endemic area for fifty-one days because of vague gastrointestinal complaints. He was completely afebrile during his course in the hospital and had a negative cutaneous reaction to coccidioidin. Eight hours after the patient was discharged from the hospital high fever and pain in the chest developed suddenly and consolidation was found in the lower lobe of the right lung. Pleural effusion subsequently developed, and the process was identified at this hospital as a coccidioidal infection. A disseminated infection, with a destructive lesion in the tenth and eleventh thoracic vertebrae developed later. The patient insists that he remained in the wards or corridors of the hospital during his entire stay. It is possible that outdoor dust containing the chlamydospores was carried into the ward by currents of air, through open windows or doors. The infection developed in March, when acute coccidioidal infection in the area is at a low ebb. According to present knowledge host to host transmission of Coccidioides immitis does not occur.[5]

The possibility of transmission of the infective agent on clothing or other articles or by contaminated dust from rooms in which patients with coccidioidal infections have been housed has been considered.[2] If infection from the latter source occurred, one must assume that the parasitic (noninfectious) form of the fungus, which is present in the sputum, had changed to the chlamydospore (or infective) form on drying in air.

Symptoms.—Though our series of cases is too small for an analysis of symptoms to have statistical value, a review of the symptoms at the onset of this disease is of interest, as the cases present a chronic and probably severe form of the infection (table 4).

The 4 patients with pneumonic onset presented a clinical picture that could not be differentiated from pneumonia of acute onset; they had temperatures of 102 to 104 F, pain in the chest, cough, toxemia and physical signs and roentgen evidence of consolidation of the type associated with

4. (*a*) Posada, A.: Un nuevo caso de micosis fungoidea con psorospermias, An. d. Circ. méd. argent. **15**:585-597, 1892. (*b*) Wernicke, R.: Ueber einen Protozoenbefund bei Mycosis fungoides (?), Centralbl. f. Bakt. (Abt. 1) **12**:859-861, 1892.

5. (*a*) Smith.[3a] (*b*) Smith, C. E.: Epidemiology of Acute Coccidioidomycosis with Erythema Nodosum, Am. J. Pub. Health **30**:600-611 (June) 1940.

lobar pneumonia. A diagnosis of lobar pneumonia was originally made for 3 of these patients. When chemotherapy produced no beneficial effect, the diagnosis was changed to primary atypical pneumonia. The 2 patients with pleural effusion exhibited an onset which differed little from that shown by patients with pneumonia. The effusion was demonstrated on the third day in one case and on the sixth day in the other. In 5 patients the onset was insidious, with cough, loss of weight, fatigue and pain in the chest. In 3 patients the disease was discovered by repeated routine cutaneous tests. Reversal of a previously negative to a positive reaction led to further study, which revealed active coccidioidomycosis. Each of the patients recalled a febrile episode accompanied by generalized aching, lassitude, cough and pain in the chest lasting a few days to two weeks, occurring after the last negative reaction to the cutaneous test with coccidioidin, though none of the patients had been sufficiently ill at the time to be hospitalized. Erythema nodosum was present early in the disease in only 2 patients in this series. It did not occur in any of the patients in whom a disseminated infection subsequently developed.

TABLE 4.—*Type of Onset in Fourteen Cases of Chronic Coccidioidomycosis*

	Number of Cases
Pneumonic	4
Pneumonic with pleural effusion	2
Insidious, with cough, weakness and slight fever	5
Unknown, infection discovered accidentally	3

From our studies we believe that there are no pathognomonic symptoms or physical signs of primary chronic coccidioidal infection. Because the clinical manifestations may be varied and bizarre, we believe that when a patient gives a history of exposure in an area in which coccidioidomycosis is endemic and presents evidence of any pulmonary disorder coccidioidomycosis should be considered as a diagnostic possibility. It should be pointed out that expectoration is usually absent or minimal during the residual or chronic phases. Cough is present, but it is usually not a prominent symptom. The pain in the chest is said to be boring and dull and to be worse at night. However, the type of pain and of cough and the amount or type of expectoration are not of any material aid in establishing the diagnosis. A clinical point of diagnostic value is the disparity between the strong roentgen evidence and the often completely lacking or minimal pulmonary or constitutional signs or symptoms in patients with cavitation. Cutaneous or osseous lesions, localized abscesses or prolonged fever may call attention to otherwise unsuspected disseminated coccidioidal infection.

Cutaneous Test with Coccidioidin.—The coccidioidin which we used for cutaneous tests was supplied to us by the Board for Investigation of Epidemic Diseases in the Army through Dr. C. E. Smith, of Stanford University, and was prepared from a number of strains of C. immitis. Fresh dilutions of coccidioidin were prepared every month, though we found even 1:1,000 dilutions to be potent six months after preparation. One-tenth cubic centimeter each of a 1:1,000 and of a 1:100 dilution were injected intradermally in separated areas on the flexor aspect of the forearm. The reaction was noted in forty-eight hours. Reactions of 0.5 cm. or more were recorded as positive as follows: 1 plus, 0.5 to 1 cm. of induration and erythema; 2 plus, 1 to 2 cm. of induration and erythema; 3 plus, induration and erythema exceeding 2 cm., and 4 plus, extensive induration, redness and edema, with vesiculation or necrosis. Induration is always present in a positive reaction. There were no untoward reactions in patients on whom cutaneous tests were performed. A review of 1 patient's record from a station hospital indicates that he had a severe local reaction and a constitutional reaction characterized by generalized aching, fever (with temperature 102 F.) and malaise of twenty-four hours' duration, beginning eighteen hours after a cutaneous test with a 1:100 dilution of coccidioidin. This patient (patient 1, table 1) was tested one month after the onset of the disease. It has been emphasized that there is no danger of producing dissemination or of reactivating an old, well focalized coccidioidal lesion by making a coccidioidin [3a] test. We have observed no parallelism between the severity of the infection and the severity of the cutaneous reaction. It has been pointed out by Smith that the cutaneous reaction to coccidioidin may be only slightly positive or may even be negative in patients with disseminated coccidioidal infection.[3a] We encountered such a condition in 1 patient. The patient (patient 13, table 1) had a 3 plus cutaneous reaction to a coccidioidin test shortly after the onset of a severe coccidioidal infection. Three weeks later, after he had manifested a septic type of fever, severe mediastinal and supraclavicular adenopathy and serologic reactions to coccidioidin indicative of dissemination, the cutaneous reaction was 1 plus. Five and six weeks after the onset of the disease the cutaneous reaction to 1:100 dilution of coccidioidin was negative. The reaction to a test made seven weeks after the onset with 1:10 dilution was also negative.

The cutaneous test with coccidioidin is of great value in the diagnosis of coccidioidomycosis. It

must be emphasized, however, that a positive reaction to this test probably has the same significance in coccidioidomycosis as a positive reaction to a tuberculin test has in tuberculosis; it indicates that the patient has or has had at some time in the past a coccidioidal infection, either clinical or subclinical, and does not necessarily imply that the patient's present illness is due to infection with C. immitis. This is well illustrated in a review of the records of coccidioidin tests made on patients in this hospital. To date, in addition to testing the personnel in our command, we have performed coccidioidin tests on 182 hospitalized patients. All of these patients had complaints referable to the lungs. Of this number 44 had positive reactions. Fourteen of the patients with positive reactions were subsequently considered to have coccidioidomycosis, while for the remaining 30 subsequent studies, including repeated examinations of sputum for C. immitis and serologic tests with coccidioidin,

TABLE 5.—*Final Diagnoses for Thirty Patients with Positive Cutaneous Reactions to Coccidioidin for Whom the Diagnosis of Coccidioidomycosis Was Not Confirmed*

	Number of Patients
Psychoneurosis	8
Asthma, bronchial	7
Tuberculosis	5
Bronchitis, chronic	4
Bronchiectasis	4
Pulmonary tumor	1
Undiagnosed	1

did not support this diagnosis. As previously noted, 15 of these 30 patients had been transferred to Hammond General hospital with the diagnosis of coccidioidomycosis. The final diagnoses are shown in table 5. The following record of a case is typical of this group.

A 25 year old air cadet was admitted to Hammond General hospital with a diagnosis of coccidioidomycosis. He had been stationed in areas in which the disease is endemic for fifteen months prior to the onset of his symptoms. He gave no history of a previous acute illness. His main complaints were headache, pain in the chest, abdominal discomfort, weakness and fatigue. Roentgen examination of the chest revealed no abnormality, and the blood count and sedimentation rate were normal. The cutaneous reaction to a 1:100 dilution of coccidioidin was positive, 2 plus. The serologic reactions to coccidioidin were negative, and C. immitis was not found in the sputum. The patient was afebrile throughout the period of hospitalization. He had many somatic complaints for which we could find no organic basis, and a psychiatric consultation confirmed our impression of psychoneurosis. The patient undoubtedly had acquired a subclinical coccidioidal infection some time during the past fifteen months, but the infection was not at all connected with or responsible for his current complaints.

It should also be mentioned that the diagnosis of coccidioidomycosis on the basis of a positive cutaneous reaction may serve to mask some more serious disorder, as exemplified by the following report of a case:

A 47 year old soldier underwent a routine roentgen examination of the chest prior to discharge from military service. The examination revealed a large, well circumscribed mass radiating from the hilus of the right lung. The patient was admitted to a station hospital, and during the course of his studies a positive cutaneous reaction to coccidioidin was obtained. On the basis of this reaction the diagnosis of coccidioidal infection was considered. On study in this hospital it was found that five years earlier he had been a resident in an area in which the disease is endemic. The serologic reactions to coccidioidin were negative. The sputum was persistently negative for C. immitis. Bronchoscopic examination revealed encroachment on the lumen of the right main bronchus by an extrinsic mass. Serial roentgen studies, including examination after injection of iodized poppy-seed oil, and repeated bronchoscopic study during the course of the next two months showed progressive increase in the size of the mass and progressive stenosis of the right main bronchus. Lateral views indicated that the mass was in the anterior mediastinum. On the basis of these findings it was thought most likely that the patient had a pulmonary tumor. Exploratory thoracotomy was suggested, but the patient refused to have the operation. We believe that this patient's positive reaction to coccidioidin was probably the result of his exposure five years previously in an area in which coccidioidomycosis is endemic and that it was not concerned with his present illness.

A positive cutaneous reaction is of special diagnostic significance in those instances in which negative reactions are known to have occurred prior to the onset of the current illness. In such a case the diagnosis of coccidioidomycosis may be made with security. We had 3 such cases in our series, in all of which the patients had coccidioidal pulmonary cavities. A report of such a case follows.

A 26 year old white officer (patient 7, table 1), formerly a resident of Denver, had been stationed in an endemic area for eighteen months. Routine tests with coccidioidin, the last being made Jan. 11, 1943, gave negative reactions. On February 14 the patient had a pain in the left side of his chest which was augmented by respiration. The pain was mild, and the patient did not report to the station hospital. During routine roentgen examination of the chest in March a cavity in the upper part of the left lung was discovered. The patient had no symptoms at that time and has had none since. The reaction to 1:1,000 dilution of coccidioidin was 3 plus. A complement fixation test made with 1:16 dilution of coccidioidin in April yielded a 2 plus reaction, and the reaction to a precipitin test was negative. Examination of the sputum in April revealed the presence of C. immitis. In this case there was no doubt of the diagnosis of coccidioidomycosis, even before the serologic reactions were reported as positive and the sputum found to contain the fungus

because the cutaneous reaction had previously been negative and was now positive.

Conversely, a negative cutaneous reaction to a test with coccidioidin generally excludes the possibility of a coccidioidal infection. It is theoretically possible that a patient with an active coccidioidal infection due to a strain not present in the coccidioidin antigen might react negatively to the test. We have not encountered such an instance, and Smith in his extensive experience has seen none. It is also possible that a patient with severe disseminated coccidioidomycosis in a late anergic phase may react negatively to coccidioidin. As previously noted, 1 such patient is included in this series. We have not observed a single patient with active primary coccidioidomycosis in whom the cutaneous reaction to a 1:100 dilution of coccidioidin has been negative. We have encountered 3 patients with negative cutaneous reaction to coccidioidin from whose sputum fungi resembling C. immitis were isolated by culture on Sabouraud's medium. Further study indicated that the fungi isolated were not C. immitis, inasmuch as they produced no lesions in guinea pigs or mice and could not be grown on special differential mediums. This aspect of the subject is further discussed in the section on cultures and inoculation of animals.

It has been stated that the cutaneous reaction to coccidioidin remains positive indefinitely in the majority of patients with coccidioidal infection.[6] From a study of repeated cutaneous tests on some of the patients in this series and from observations made on personnel in this command it is thought that the sensitivity to coccidioidin may diminish and may under some circumstances disappear. These observations were included in another report.

The absence of cross sensitivity between coccidioidin and tuberculin has been demonstrated.[7] Of the 44 patients in whom the cutaneous reaction to coccidioidin was positive, 29 were tested with tuberculin. In 16 of these patients the reaction was negative. We observed 5 patients for whom the diagnosis of active tuberculosis was definitely established and who were also sensitive to coccidioidin. All of these patients gave a history of being quartered in an area in which coccidioidomycosis is endemic. Studies of the sputum and serologic tests with coccidioidin showed no evidence of active coccidioidomycosis in these patients. Of our 14 patients with definite coccidioidal infection, 8 reacted negatively to tuberculin. We have seen no patient in whom both active tuberculosis and active coccidioidomycosis were simultaneously present, though this has been encountered.[8] It has been pointed out that the use of separate syringes for testing with tuberculin and with coccidioidin is necessary to prevent false positive readings.

Emmons and Ashburn have shown that some cross sensitivity exists for a fungus that they isolated from rodents in Arizona, which has been termed Haplosporangium parvum.[9] Smith observed that persons who live in some areas in the Middle West, such as Ohio, Indiana, Michigan, Illinois and Missouri, may show a borderline positive reaction to coccidioidin, which he conjectures may be due to infection with some other fungus.[3a] Despite these observations the test may be considered highly specific, indicating coccidioidal infection, past or present. Smith has emphasized that a positive cutaneous reaction to coccidioidin also indicates immunity to exogenous infection.[10]

Culture of Organisms and Inoculation of Animals.—Definite proof of the existence of coccidioidal infection is obtained when the organism is grown on culture and on subsequent inoculation into a guinea pig or mouse produces the characteristic spherules in the tissues of the animal. In cases of disseminated coccidioidomycosis with draining sinuses or superficial abscesses this is usually readily accomplished. In cases of primary pulmonary coccidioidomycosis, however, recovery of the fungus is more difficult, largely because patients with an infection of this type usually raise little sputum. Nevertheless repeated routine cultures of the sputum, or of gastric washings if necessary should be made. If the latter material is used, the culture should be made rapidly, because the gastric juice digests the fungus. Material obtained at bronchoscopy from 1 patient in this series who raised no sputum yielded positive results on culture and inoculation of animals. It has been pointed out by others that routine studies of sputum are not indicated in cases of coccidioidomycosis, since the diagnosis may be more readily made by other means. This may be true of the early

6. (*a*) Coccidioidomycosis Control Program for the Army Air Forces Western Flying Command.[2] (*b*) Smith.[5b]

7. (*a*) Kessel, J. F.: The Coccidioidin Skin Test, Am. J. Trop. Med. **19**:199-204 (March) 1939. (*b*) Aronson, J. D.; Saylor, R. M., and Parr, E. I.: Relationship of Coccidioidomycosis to Calcified Pulmonary Nodules, Arch. Path. **34**:31-48 (July) 1942.

8. Smith, C. E.: Personal communication to the authors.

9. Emmons, C. W., and Ashburn, L. L.: Isolation of Haplosporangium Parvum, N. sp., and Coccidioides Immitis from Wild Rodents: Their Relationship to Coccidioidomycosis, Pub. Health Rep. **57**:1715-1729 (Nov. 13) 1942.

10. Smith, C. E.: Parallelism of Coccidioidal and Tuberculous Infections, Radiology **38**:643-648 (June) 1942.

stages of acute infections, during which the serologic reactions to coccidioidin are usually positive. We believe that studies of the sputum may be especially helpful in establishing the diagnosis of chronic primary coccidioidomycosis, in which the serologic reactions may be negative.

It should be emphasized that diagnosis of coccidioidal infection by means of examination of direct smears is an unreliable method and should not be used. C. immitis will grow on all mediums used in routine procedures. In this series Sabouraud's medium and a differential medium devised in the Stanford University laboratory by Smith and his co-workers were used for culture. The latter medium contains 1 per cent of ammonium chloride, 1 per cent of sodium acetate, 0.8 per cent of tribasic potassium phosphate and 2 per cent of agar, with 0.04 per cent of cupric sulfate added after the medium is autoclaved. Although Coccidioides grows scantily on this, few other fungi show any growth at all. In 4 instances in which we isolated a fungus that on Sabouraud's medium produced a white, cottony growth [3b] resembling C. immitis, the fungus showed no growth whatsoever on the differential medium and subsequent inoculation of animals produced no coccidioidal lesions. We have found this medium to be of considerable diagnostic value. The following report of a case illustrates this point:

A 41 year old man had been stationed in an endemic area for nine months when cough, profuse expectoration, loss of weight and a slight afternoon fever developed. Roentgen examination of the chest showed no evidence of parenchymal infiltration. A cutaneous test with a 1:100 dilution of coccidioidin made three months after the onset of the illness, resulted in a 2 plus reaction. The serologic reactions to coccidioidin were negative. A white cottony growth apparently typical of C. immitis was found on 2 cultures of the sputum on Sabouraud's medium. Subculture of this fungus on the differential medium produced no growth. Inoculation of animals produced no evidence of the presence of C. immitis. Roentgen examination after injection of iodized poppyseed oil subsequently revealed the presence of moderate bronchiectasis. In view of these findings, it was felt that the fungus isolated from the sputum of the patient was definitely not C. immitis.

As pointed out previously inoculation of animals should be done in every instance to establish the diagnosis incontrovertibly. Saline suspensions of the fungus may be inoculated intraperitoneally in a mouse or intratesticularly in a guinea pig. Peppler [11] suggested subcutaneous inoculation in the groin of the guinea pig, but we have not used this method. Mice usually die the second week after inoculation, and guinea pigs may be killed after three or four weeks. Smith [3a] suggested treating sputum with 0.05 per cent cupric sulfate before inoculation, though pleural fluid or uncontaminated pus may be inoculated directly. We have followed the practice of making initial cultures of the suspected material. If these are suggestive, the material is then inoculated into animals, usually mice. The characteristic nonbudding spherules with a double refractile wall and containing endospores must be demonstrated in tissue sections from the animal. C. immitis was isolated in 6 of the cases of primary coccidioidomycosis and in all of the cases of disseminated coccidioidomycosis in this series. Subcultures of the organisms isolated were sent to Dr. C. E. Smith, who confirmed their identity as C. immitis. The following report of a case presents an interesting example of the need for careful combined studies by cultures and by inoculation of animals:

A 26 year old white man had been a resident of an area in which coccidioidomycosis is endemic until he was 14 years of age. For six years he had had a mild cough and occasional blood-streaked sputum. For three months he complained of cough, pain in the chest and hemoptysis. Roentgen examination of the chest revealed no abnormalities. The cutaneous reaction to a 1:1,000 dilution of coccidioidin was positive, 2 plus. The serologic reactions to coccidioidin were negative. On three occasions a white, cottony growth characteristic of C. immitis was obtained by culturing sputum on Sabouraud's medium. Subculture of this fungus on differential mediums produced no growth, and the results of inoculation of animals were negative. A culture of the fungus was sent to Dr. C. E. Smith, who corroborated our impression that the organism was not Coccidioides and who prepared an antigen for cutaneous testing from this culture. A cutaneous test with a 1:100 dilution of this antigen produced a 3 plus reaction in the patient. Cutaneous tests with this antigen were made on 5 patients with known positive cutaneous reactions to coccidioidin in order to rule out cross sensitivity. All of the patients reacted negatively. Cutaneous tests were also made on 8 patients who had negative cutaneous reactions to coccidioidin and on 2 other patients who had an atypical fungus in their sputum. In all of these patients the reaction was negative. Because this patient had a positive cutaneous reaction for this fungus, we think that possibly it had a casual relationship to his illness. In this instance less careful studies would have easily led to the erroneous diagnosis of coccidioidomycosis.

Serologic Tests with Coccidioidin.—We have found determination of the serologic reactions to coccioidin to be a valuable diagnostic and prognostic aid in chronic coccidioidal infection. In these tests coccidioidin is used as an antigen and the patient's serum is tested for complement-fixing antibodies and precipitin. The tests were performed for us by Dr. C. E. Smith and Miss R. J. Wheatlake, under the Board for the Investigation of Epidemic Diseases in the Army.

11. Peppler, H. J.: Routine Procedures for the Isolation and Identification of Pathogenic Fungi, Am. J. Clin. Path. **13**:123-127 (Nov.) 1943.

Briefly, it may be stated that in the early stages of acute infections, except those that are exceptionally mild, the reactions to both the complement fixation test and the precipitin test may be positive. Usually in the early stages the precipitins are present in higher dilutions than the complement-fixing antibodies. The precipitins, however, usually disappear earlier in the course of the disease than the complement-fixing antibodies. In general the severer the infection the greater the possibility that the reactions will be positive. With focalization of the infection, such as exists in patients with pulmonary cavities, the antibodies may rapidly disappear or the complement-fixing antibodies may persist in low dilutions. Conversely, in patients with disseminated lesions or with impending dissemination the titer of the complement-fixing antibody rises. Thus the serologic tests with coccidioidin are not only of diagnostic value but of considerable prognostic significance in indicating the course of the disease in a given patient. Serial serologic tests also lend support to the contention that disseminated infection usually occurs as a disease continuous with primary infection and that no sharp delineation exists between them.

From a study of the patients reported on in this paper, we believe that a diagnosis of disseminated coccidioidomycosis should rarely, if ever, be made in the absence of specific antibodies in relatively high dilutions even though other data may point to this diagnosis. In several instances in which we ventured the diagnosis of disseminated coccidioidomycosis for patients with positive cutaneous reactions to coccidioidin and with negative serologic reactions the correct diagnosis ultimately proved to be some condition other than coccidioidal infection. The following report of a case illustrates this point.

A 37 year old Filipino private who had been a resident of an endemic area for the past ten years was admitted to a station hospital complaining of pain and stiffness in the neck. Roentgen examination of the neck and chest disclosed no abnormalities, but it was discovered that the patient's temperature rose to 102 F. daily. During the course of observation positive cutaneous reactions to both coccidioidin and tuberculin were observed. Approximately five weeks after the patient entered the station hospital, roentgen examination of the chest showed localized destructive lesions in the first rib on the left and the third rib on the right. A biopsy was made from one of these areas, and the specimen was reported as showing chronic giant cell granulomatous tissue the cause of which could not be determined from microscopic study. No cultures were made at the time of biopsy. The patient was transferred to Hammond General Hospital approximately two months after the onset of the illness. Repeated examinations of the sputum revealed no tubercle bacilli. Serial roentgenograms showed increasing areas of destructive lesions in the right humerus, both scapulas and the pelvis. There was no evidence of involvement of the pulmonary parenchyma. The serologic reactions to coccidioidin were negative on two occasions. The patient was seen by Dr. C. E. Smith, who thought that because the serologic reactions were negative a diagnosis of disseminated coccidioidomycosis, which we had considered, was not likely. A biopsy was again made a month after the patient entered Hammond General Hospital and again revealed chronic granulomatous tissue with no indication as to the causation. However, cultures were made at the time of biopsy on Wallenstein's medium for Mycobacterium tuberculosis and on Sabouraud's medium for Coccidioides. The culture for M. tuberculosis was positive. The patient died six months after the onset of the disease, and autopsy revealed widespread tuberculous infection involving particularly the bones. In this case the negative serologic reactions to coccidioidin indicated that the condition was not disseminated coccidioidomycosis, even though the clinical picture was highly suggestive of this disease.

It must be borne in mind that negative serologic reactions do not rule out the diagnosis of primary coccidioidal infection. Of the 14 patients we observed, 9 had positive serologic reactions. Four of these had disseminated infections. In 3 of these 4 patients we were able to observe a definite fall in the titer of the coccidioidal antibodies coincident with the clinical improvement of the patients and as the infections became focalized. The remaining 5 patients with positive serologic reactions had primary infections. In 2 of these patients pulmonary cavities were present. The titer was highest in 2 patients with progressive coccidioidal infection; both of them showed 4 plus complement fixation with a 1:32 dilution of the antigen.[11a] In 2 patients who were clinically well and who had normal sedimentation rates for four and five months respectively the complement-fixing antibodies were still present. In 1 of these patients the reaction was positive with a 1:4 dilution of coccidioidin thirteen months after the onset of the disease, and in the other it was positive with a 1:2 dilution nine months after the onset. Precipitins were absent from the serum of both of these patients.

Studies of Tissues.—Demonstration of spherules in sections of tissues removed at biopsy is often difficult. The characteristic nonbudding spherules containing endospores must be demonstrated. Frequently, the problem of differentiation between tuberculosis and coccidioidal infection will arise when a biopsy is made and when microscopic examination is done. In such in-

11a. We have since observed 2 additional patients with disseminated coccidioidal infection whose complement fixation was 4 plus. The titer dropped slightly in one of these patients five months after the onset and in the other six months after the onset, as clinical improvement was occurring.

stances we found it especially advantageous at the time of biopsy to make cultures, on Wallenstein's medium for M. tuberculosis and on Sabouraud's and differential mediums for Coccidioides and in selected cases to inoculate guinea pigs or mice. We had 3 cases in which we were confronted with this problem. In all of them the cutaneous reactions to both tuberculin and coccidioidin were positive. A culture made at the time of biopsy on 1 of the patients yielded C. immitis. A culture made for the second patient at the time of biopsy showed M. tuberculosis, though by examination of the tissue alone, as previously stated, differentiation between coccidioidal and tuberculous infection was impossible. The third patient presented the clinical picture of diffuse osseous involvement and extensive pulmonary infiltration. Two biopsies of the diseased bone revealed fibrous osteitis, which is probably indicative only of rapid decalcification of bone and is probably not of etiologic significance. Cultures made at biopsy were negative for Coccidioides and M. tuberculosis. The serologic reactions to coccidiodin were negative on

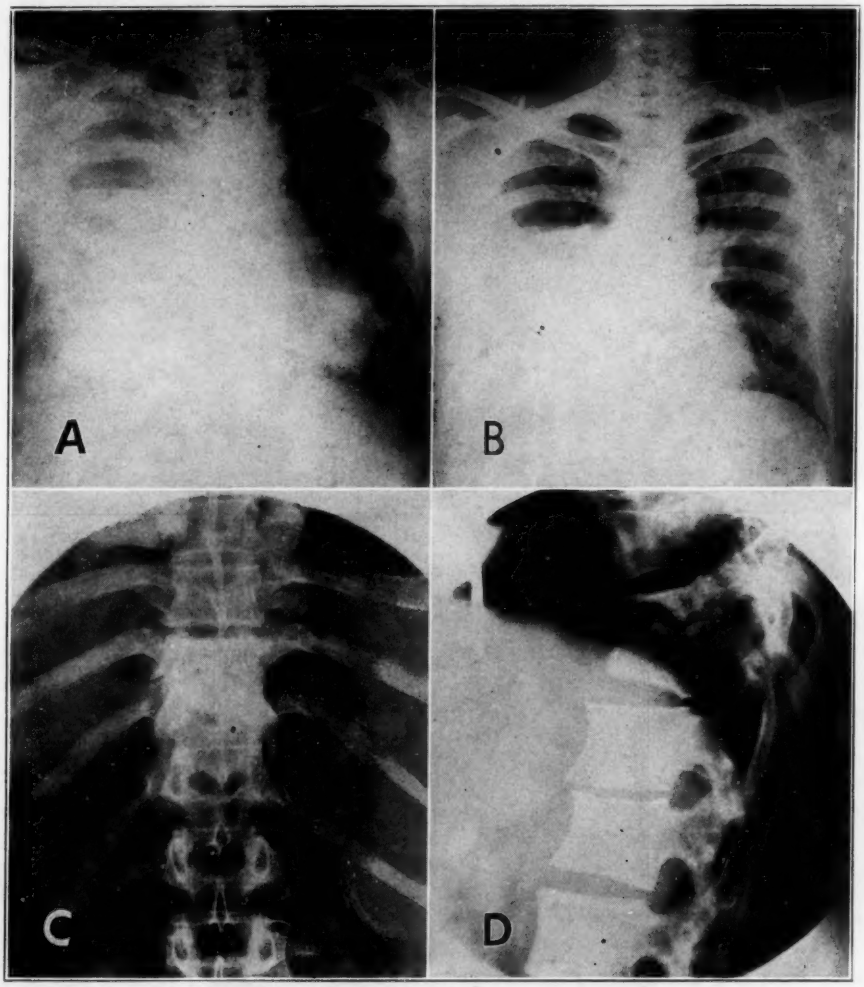

Fig. 1 (patient 12, table 1).—Roentgenograms of a 31 year old Mexican with disseminated coccidioidomycosis of acute pneumonic onset, with pleural effusion. The cutaneous reaction to coccidioidin was positive, and the sputum contained C. immitis. The serologic reactions were positive with coccidioidin in high titer. The patient had low grade fever for five months and continued to have a rapid sedimentation rate following this. *A*, taken ten days after the onset of the illness, shows an extensive effusion in the right side of the chest. *B*, taken ten months later, shows uniform density of the lower half of the right pulmonary field, extending up to the interlobar pleura. *C* and *D*, made eight and a half months after the onset, show destruction of the tenth thoracic vertebra with some erosion of the eleventh; the intervertebral disk is destroyed. The patient was asymptomatic; the serologic reactions to coccidioidin had decreased, and the sedimentation rate was normal.

two occasions. Early in the course of the illness coccidioidomycosis was strongly considered, and later it was thought that the patient had tuberculosis. At present the diagnosis is still in doubt, though on the basis of the results of culture of material obtained at biopsy it is not likely that either of these conditions is present.[11b]

Studies of the Blood.—We have been unable to observe any significant changes in the white blood cell counts of patients with chronic coccidioidal infection. Eosinophilia has been reported in early coccidiodomycosis.[12] Review of the records of our patients with chronic infection reveals that in the early stages the number of white cells per cubic millimeter of blood ranged

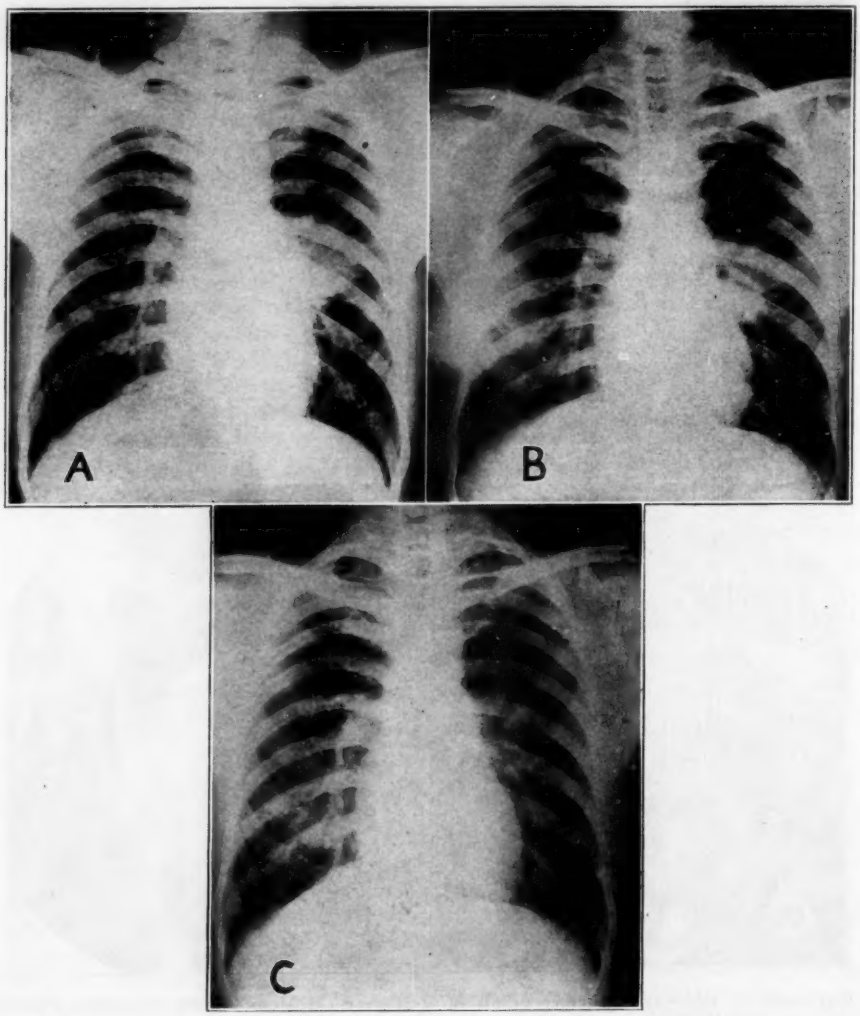

Fig. 2 (patient 1, table 1).—Roentgenograms of a 32 year old Filipino with disseminated coccidioidomycosis of acute pneumonic onset, with positive cutaneous and serologic reactions to coccidioidin and with sputum repeatedly positive for C. immitis. *A*, taken three weeks after the onset of the illness, shows a pneumonic area radiating from the hilus of the left lung; *B*, taken ten weeks after the onset, shows a striking decrease in the area of density with a suggestion of cavitation. At this time the patient complained of pain in the left side of the chest posteriorly; he had low grade fever, moderate cough and scant expectoration. *C*, taken at the time of discharge, eight months after the onset, shows advanced clearing of the density; no cavity was demonstrated; small areas of patchy infiltration remain near the hilus. At this time the patient had no symptoms and was discharged to duty.

11b. This patient died eleven months after the onset, and autopsy revealed generalized carcinomatosis arising from a very small adenocarcinoma of the pancreas.

12. (*a*) Goldstein and Louie.[1d] (*b*) Smith.[3a]

from 6,000 to 20,000. The differential counts for the patients with leukocytosis showed an increase in the proportion of polymorphonuclear leukocytes to 70 to 80 per cent. There were no statements on the early hospital records indicating that an increase in the number of young forms of polymorphonuclear cells was encountered. One patient had eosinophilia (eosinophils constituting 10 per cent of the leukocytes) early in the course of the disease. It is possible that more frequent determinations early in the illness might have revealed a higher incidence of eosinophilia.

The sedimentation rate is increased during the stage of active infection. In general it parallels other evidence of active disease. The fall to normal usually develops several weeks to months after the temperature has subsided. In the patients we observed the sedimentation rate usually dropped several months before the titer of the complement-fixing antibodies diminished. Of our 5 patients with cavities only 1 had a rapid sedimentation rate on entry to this hospital. A persistently rapid sedimentation rate is to be given serious attention, as is noted from an analysis of the following report of a case.

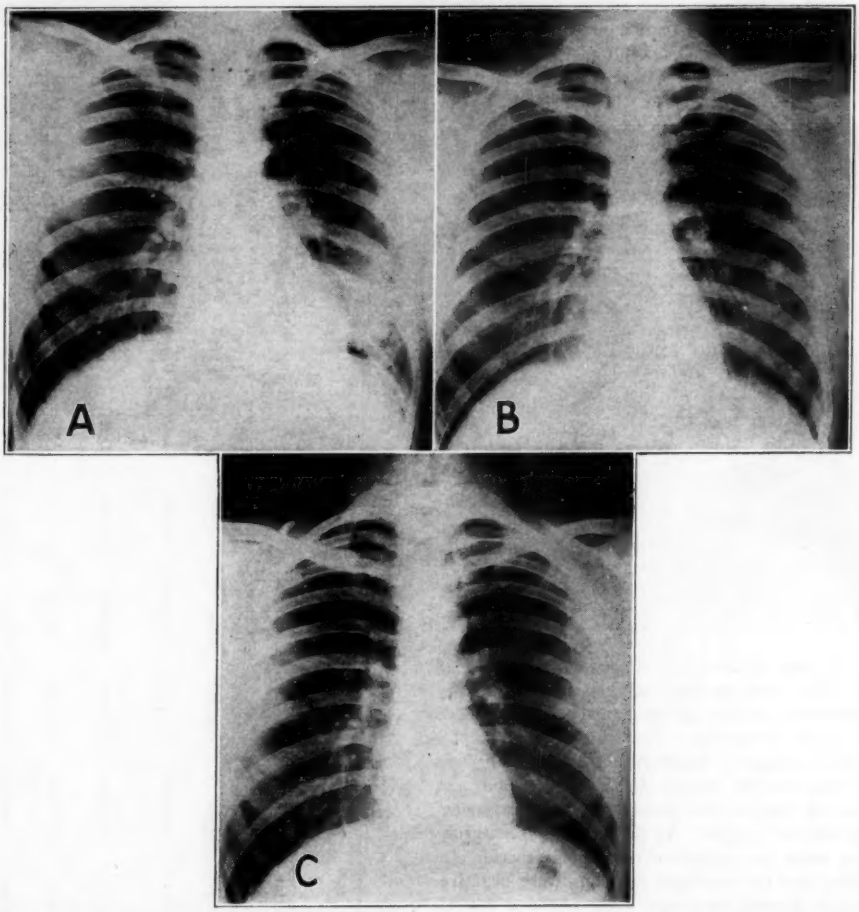

Fig. 3 (patient 2, tables 1 and 6).—Roentgenograms of a 25 year old white man with primary coccidioidomycosis of acute pneumonic onset. The patient's cutaneous reaction to coccidioidin was positive, and his sputum repeatedly gave cultures which were positive for C. immitis. The serologic reactions were positive with coccidioidin in high dilutions, and the complement fixation reaction was still positive (4 plus) with a 1:4 dilution ten months after the onset of the illness. *A*, taken one month after the onset shows a large pneumonic area in the lower lobe of the left lung. The density gradually cleared, and the patient was discharged to duty five months later. *B*, made eight months after the onset of the illness, shows a cavity in the lower lobe of the left lung. *C*, made at time of discharge, thirteen months after onset and one month after phrenicotripsy, the pulmonary fields are clear, with no evidence of a cavity. The patient was asymptomatic.

A 31 year old Mexican (patient 12, table 1) had acute coccidioidal infection with pneumonia and pleural effusion. He had low grade fever that persisted for five months. Following this, the sedimentation rate remained persistently rapid, despite the normal temperature and the clearing of the density in the chest as shown by serial roentgenograms. Eight months after the onset of the illness the patient complained of backache and roentgen examination revealed a destructive lesion of the tenth and eleventh thoracic vertebrae

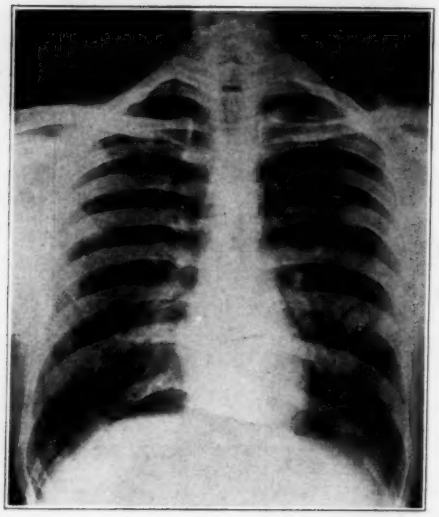

Fig. 4 (patient 4, table 1).—Roentgenogram of a 28 year old white man showing solitary nodular density in the lower lobe of the left lung, diagnosed as healed primary coccidioidomycosis. The patient was asymptomatic. His cutaneous reaction to coccidioidin was negative nine months before the roentgenogram was made; in the interim the patient was on maneuvers in highly infected terrain. At the time the roentgenogram was made the cutaneous reaction to coccidioidin was positive and the serologic reactions were negative. Sputum and gastric washings were negative for C. immitis and for M. tuberculosis. The cutaneous reaction to tuberculin was negative. The patient returned to duty.

(fig. 1). This patient's serologic reaction with coccidioidin was corroborative, being positive, 4 plus with a 1:32 dilution of the antigen. The persistently rapid sedimentation rate in this patient was a definite indication of impending trouble, despite the lack of fever and of clinical signs.

Of course, a rapid sedimentation rate and a positive cutaneous reaction to a coccidioidin test do not necessarily imply that the coccidioidal infection is the cause of the rapid rate.

Roentgenologic Studies.—The early pulmonary roentgen observations in coccidioidal infection

TABLE 6.—*Data for Patients with Primary Coccidioidal Pulmonary Cavities*

Patient, No. in Table 1	Roentgen Findings at Onset	Time Between Onset of Illness and Discovery of Cavity, Mo.	Fever Since Entry to Hospital	Chest Symptoms in Hospital	Cutaneous Reaction Previous to Onset of Illness	Cutaneous Reaction to Coccidioidin	Culture of Sputum for Coccidioides	Serologic Reaction to Coccidioidin	Erythrocyte Sedimentation Rate	Location of Cavity: Lung	Location of Cavity: Lobe	Diameter of Cavity, Cm.	Length of Time Cavity Persisted After Discovery Mo.	Treatment	Comment
5	Mottled density, upper lobe of left lung	2	None	None	Unknown	Positive	Negative	Negative	Normal	Left	Upper	4	3	Rest in bed	Patient discharged from service because of age (44)
3	Infiltration, upper lobe of left lung	12	None	Cough, sputum for a few days	Negative	Positive	Negative	Negative	Normal	Left	Upper	3.5	9	Rest in bed; shot bag; phrenicolysis	Cavity smaller; patient returned to duty
7	.....	2	None	None	Negative	Positive	Positive	Positive	Normal	Left	Upper	1.5	8	Rest in bed; shot bag	Cavity disappeared; fibrosis remained; patient returned to duty
2	Consolidation, lower lobe of left lung	7	None	Pain in chest at night	Unknown	Positive	Positive	Positive	Normal	Left	Lower	1.5	7	Rest in bed; phrenicolysis	Cavity disappeared 3 weeks after phrenicolysis; patient returned to duty
8	.....	3	None	None	Negative	Positive	Negative	Negative	Rapid at present, 7 mo. after discovery of cavity	Right	Upper	2	7	Rest in bed; shot bag	Cavity has decreased in size; patient still in hospital at time of report

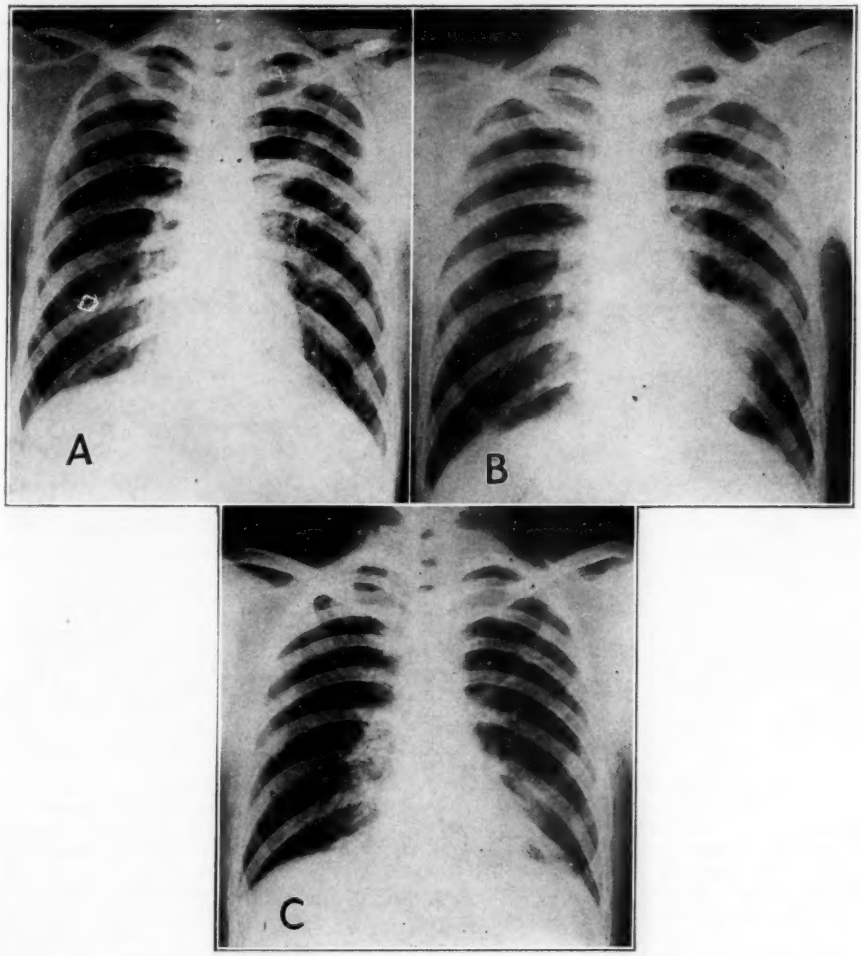

Fig. 5 (patient 3, tables 1 and 6).—Roentgenograms of a 28 year old white man with primary coccidioidomycosis of insidious onset, with cough and loss of weight. The patient had a negative cutaneous reaction to coccidioidin four months before the onset of the illness; since he entered the hospital his reaction has been positive. The serologic reaction to coccidioidin thirteen months after the onset was negative. Sputum and gastric washings were negative for C. immitis. The cutaneous reaction to tuberculin was negative, and repeated studies of the sputum and inoculations of guinea pigs failed to demonstrate the presence of M. tuberculosis. *A*, taken two weeks after the onset of the illness, shows patchy infiltration of the upper lobe of the left lung. The patient was returned to duty after one month and remained asymptomatic for one year. *B*, taken one year after the onset, shows a large thin-walled cavity in the upper lobe of the left lung, with fibrotic strands extending downward from the cavity to the hilus, a pneumonic area along the left border of the heart and small areas of patchy infiltration scattered throughout the upper half of the left lung and in the right infraclavicular area. *C*, taken five months after *B*, shows considerable clearing of the pulmonary fields. The cavity is somewhat smaller, and the area of increased density along the left border of the heart is small. The patient was entirely asymptomatic. Phrenicolysis was performed. The patient returned to duty.

have been described in the literature.[13] Briefly, they may range from simple hilar thickenings to pneumonic infiltrations or massive effusions. The pneumonic infiltrations may resemble atypical lobar pneumonia or bronchopneumonia (figs. 2 and 3). Solitary nodular parenchymal

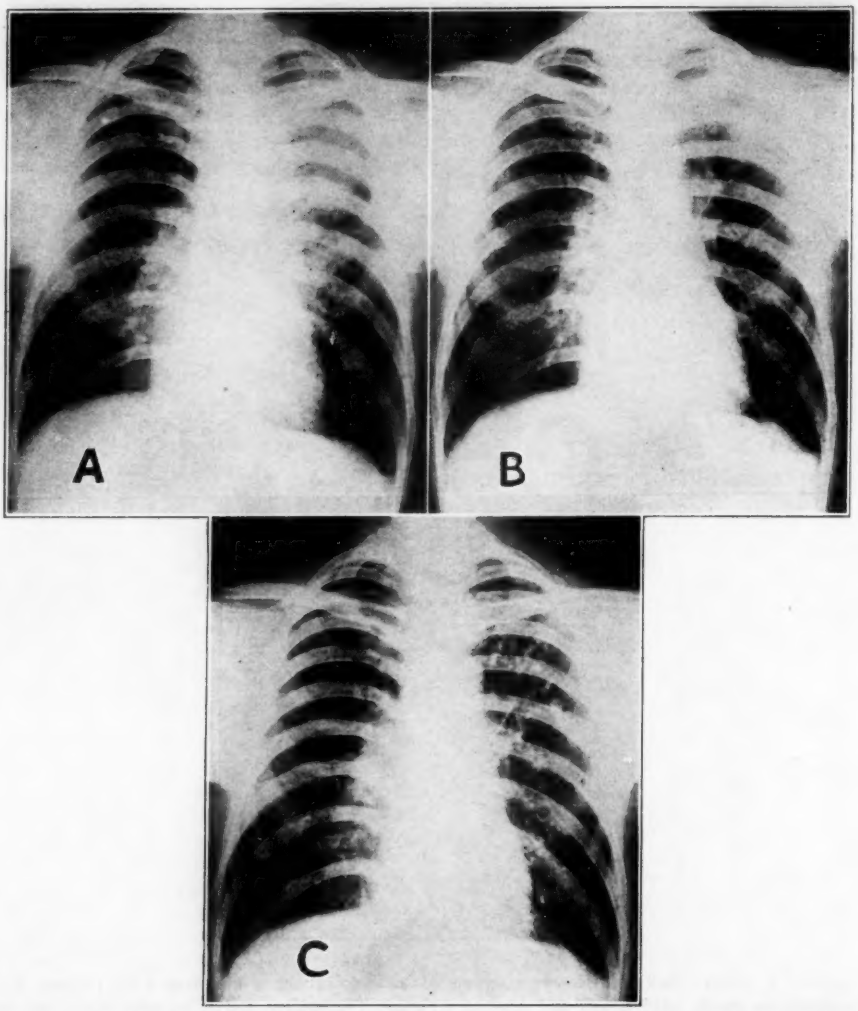

Fig. 6 (patient 5, tables 1 and 6).—Roentgenograms of a 44 year old white man with primary coccidioidomycosis of insidious onset, with cough, weakness and pain in the chest. The cutaneous reaction to coccidioidin was positive, but the sputum and gastric washings were negative for C. immitis, and the serologic reactions to coccidioidin were negative. The sputum was repeatedly negative for M. tuberculosis. *A*, taken one month after the onset of the illness, shows mottled infiltration of the upper two thirds of the left lung and a moderately thick-walled cavity in the left infraclavicular region. *B*, taken eight days later shows considerable clearing of the patchy infiltration. The cavity is the same in diameter. *C*, taken three months after *B*, shows a large, thin-walled cavity in the apex of the left lung. Iodized poppyseed oil did not enter the cavity. The patient was asymptomatic at this time.

lesions may occur and may resemble malignant metastases. The solitary densities may undergo central cavitation and produce a cystlike cavity, or they may become fibrous and undergo calcification (fig. 4). In some instances the infiltrations are apparently mainly exudative in character and clear in a few weeks. In others they

13. (*a*) Coccidioidomycosis Control Program for the Army Air Forces Western Flying Command.[2] (*b*) Powers, R. A., and Starks, D. J.: Acute (Primary) Coccidioidomycosis: Roentgen Findings in a Group "Epidemic," Radiology **37**:448-453 (Oct.) 1941. (*c*) Dickson, E. C., and Gifford, M. A.: Coccidioidomycosis: Primary Type of Infection, Arch. Int. Med. **62**:853-871 (Nov.) 1938.

appear to be productive and clear slowly and incompletely, leaving residual fibrosis. We believe that there is no early roentgen evidence that is pathognomonic of coccidioidal infection. It should be pointed out also that in some primary coccidioidal infections no pulmonary change may be demonstrable by roentgen examination.

A characteristic but not pathognomonic roentgen evidence of chronic coccidioidal infection is the presence of a solitary, thin-walled, balloon-like cavity with little or no surrounding reaction (figs. 3, 5, 6 and 7). Such a cavity forms in the site of earlier pneumonic infiltrations or nodular densities, sometimes after a latent period and in determining its contour. By correlation of roentgenography with the diagnostic procedures outlined in the preceding paragraphs the presence or absence of coccidioidal infection in a given patient who has a suspicious roentgen picture can be determined.

The primary coccidioidal cavity is perhaps most common in the upper lobe, though it may occur anywhere in the lung. Table 6 shows a more detailed analysis of the conditions of 5 patients with cavities. The data given in this table illustrate the silent asymptomatic character of the cavity, the benign course of the illness, the tendency of the cavity to persist and the

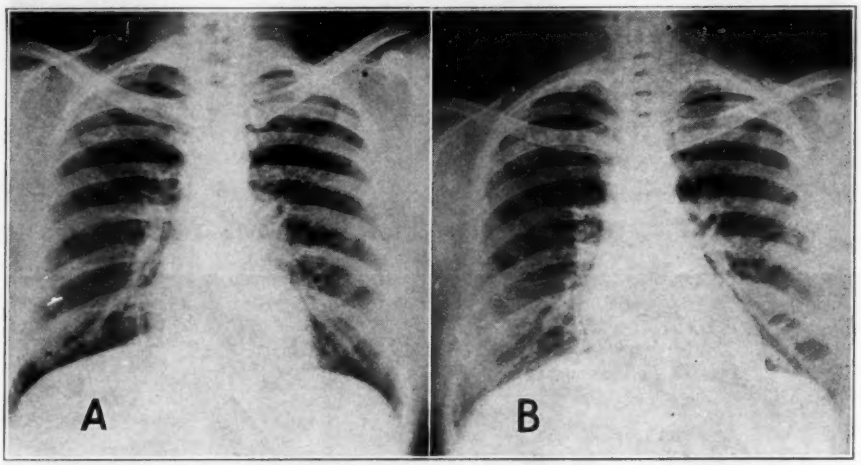

Fig. 7 (patient 8, tables 1 and 6).—Roentgenograms of a 31 year old white man with primary coccidioidomycosis. The last negative cutaneous reaction to coccidioidin was obtained three months earlier. The patient had had a febrile illness two months before the first roentgenogram was made, with pain in the chest, cough and fever of two weeks' duration. When the patient entered the hospital the cutaneous reaction to coccidioidin was positive but the serologic reaction was negative and the sputum and gastric washings were negative for C. immitis. The sedimentation rate was rapid. The patient had no symptoms. *A*, taken two months after the onset of the illness, shows a small cavity in the right infraclavicular area. In *B*, taken seven months after the onset, the cavity is slightly smaller.

during which roentgenograms of the chest are normal. A striking feature is the tendency for the cavity to persist despite regression of other associated pulmonary lesions. When such a cavity is observed roentgenographically coccidioidal infection should be suspected. A similar roentgenographic appearance may, however, be produced by several other conditions, including acute pulmonary infections, tuberculosis, pyogenic infections, necrosis of malignant tumors, emphysematous pleural blebs, localized pneumothoraces and congenital or acquired pulmonary cysts.[14] Lateral, oblique and stereoscopic views are helpful in accurately localizing the annular shadow

14. Sante, L. R., and Hufford, C. E.: Annular Shadows of Unusual Type Associated with Acute Pulmonary Infection, Am. J. Roentgenol. **50**:719-732 (Dec.) 1943.

absence of spread of pulmonary lesions such as would be expected in a patient with a tuberculous cavity. The apparent resistance both to exogenous superinfection and to endogenous reinfection in patients exhibiting pulmonary cavities is striking. In 1 patient we attempted to fill the cavity with iodized poppyseed oil but were unsuccessful (fig. 6). This procedure is, however, rarely indicated or necessary. Winn[15] has emphasized that such a large, thin-walled cavity may be of mechanical origin, resembling a tension cyst, rather than due to necrosis and excavation of pulmonary tissue. In 1 patient (patient 1, table 1) there were rarified areas present in a dense consolidation, a condition

15. Winn, W. A.: Pulmonary Cavitation Associated with Coccidioidal Infection, Arch. Int. Med. **68**:1179-1214 (Dec.) 1941.

which the roentgenologist interpreted as multiple cavitation. The areas subsequently cleared (fig. 2). Cavities of this type may be due to tissue necrosis and are totally different from those in other patients in the series.

infiltration and miliary dissemination. Mediastinal adenopathy has also been pointed out as a frequent outstanding characteristic of coccidioidal infection of this type (figs. 9 and 10),[16] and attention has been directed to its ominous prog-

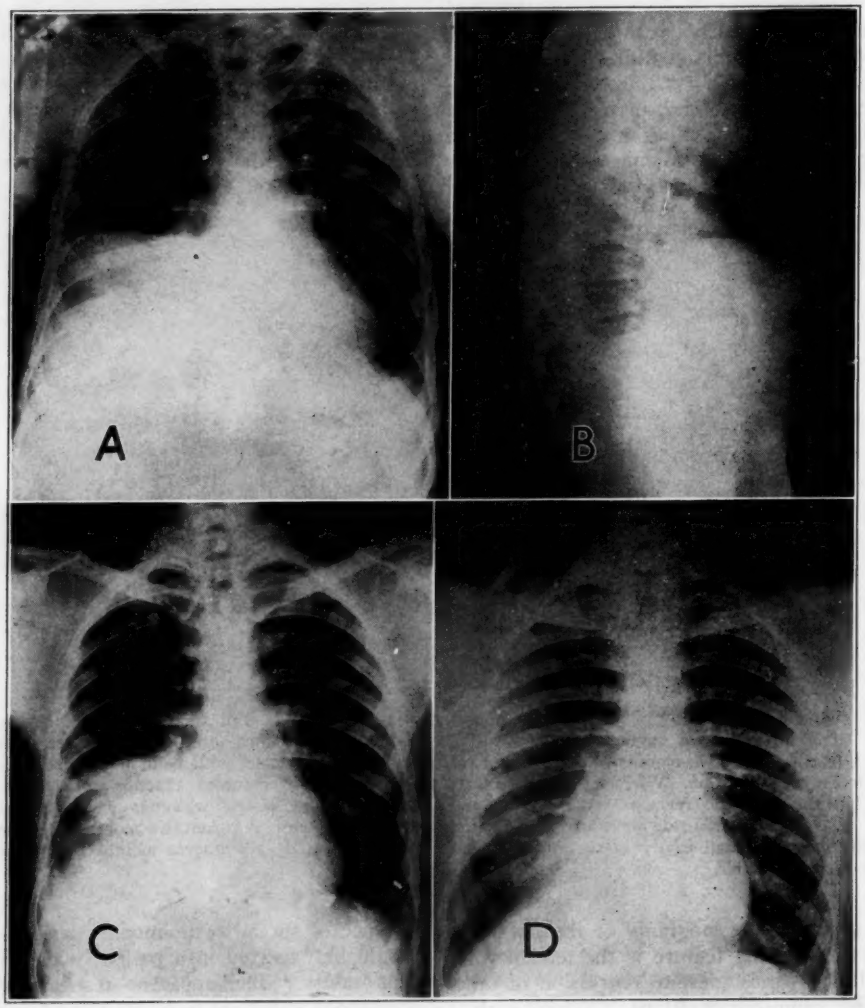

Fig. 8 (patient 9, table 1).—Roentgenograms of a 28 year old white man with primary coccidioidomycosis of acute pneumonic onset. The cutaneous and serologic reactions to coccidioidin were positive, and sputum obtained at bronchoscopy contained C. immitis. *A* and *B*, taken one week after the onset of the illness, show extreme homogeneous density of the lower lobe of the right lung. *C*, taken three weeks after the onset, shows some clearing at the periphery. *D*, taken six months after the onset, shows a triangular shadow in the lower lobe of the right lung. At this time the patient was asymptomatic and had a falling sedimentation rate and decreasing serologic reactions to coccidioidin.

We encountered 1 patient who presented the picture of collapse of the lower lobe and in whom we were unable to determine any intrinsic or extrinsic obstruction to the bronchus (fig. 8).

The most common pulmonary roentgen evidences of disseminated coccidioidomycosis are marked progressive pneumonic consolidation and

nostic significance. The conditions observed roentgenologically in bones and joints in dis-

16. (*a*) Coccidioidomycosis Control Program for the Army Air Forces Western Flying Command.[2] (*b*) Winn, W. A., and Johnson, G. H.: Primary Coccidioidomycosis: A Roentgenographic Study of Forty Cases, Ann. Int. Med. **17**:407-422 (Sept.) 1942.

seminated coccidioidal infection have been studied by Carter.[17] They may consist of cystlike, sharply circumscribed osteolytic lesions with little reaction surrounding them. The lesions may be multiple and are especially apt to occur in cancellous bone. Differentiation of coccidioidomycosis from tuberculosis in an individual lesion is rarely possible. Figure 1 shows destructive lesions of thoracic vertebrae in a patient with disseminated coccidioidal infection.

It is also of interest that in each patient with primary chronic coccidioidal infection a parenchymal lesion was demonstrable roentgenologically. Of 8 patients with positive reactions to coccidioidin and chronic pulmonary symptoms but with no roentgenographic evidence of lesions in the chest for whom the diagnosis of coccidioidomycosis was strongly considered, for not 1 was the diagnosis substantiated. One of these patients had an atypical fungus in the sputum, as described previously.

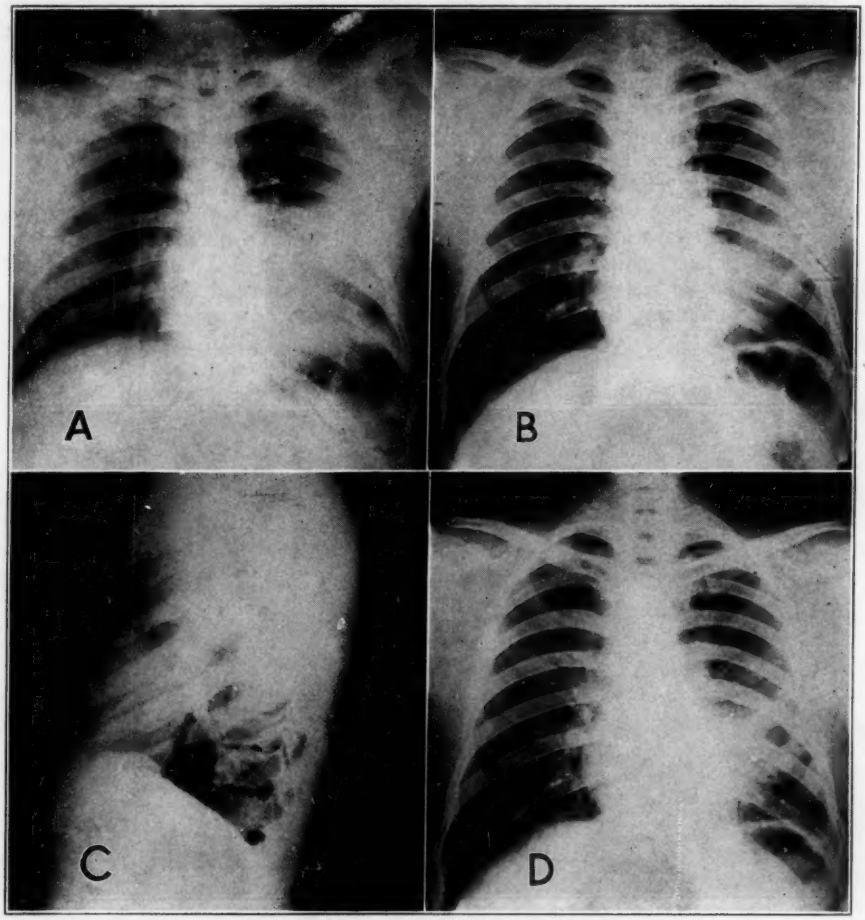

Fig. 9 (patient 11, table 1).—Roentgenograms of a 26 year old Mexican with disseminated coccidioidomycosis of acute pneumonic onset, with pleural effusion. The cutaneous reaction to coccidioidin was positive. The sputum was negative for C. immitis but fluid from a mediastinal abscess was positive. The serologic reactions to coccidioidin in dilutions in the range used for disseminated coccidioidomycosis were positive. *A*, taken five days after the onset of the illness, shows a diffuse pneumonic area in the lower part of the left pulmonary field. *B*, taken five and a half months after the onset, shows partial clearing of the pneumonic area, and extensive widening of the superior mediastinum. *C*, a lateral view, shows the outlines of soft tissue masses above and beneath the sternum. *D*, taken seven and a half months after the onset, at the time of transfer to a veterans' hospital, shows a decrease in the density of the chest and a decrease in the size of the mediastinum. The patient was afebrile at this time; he had a draining sinus over the abscess.

17. (*a*) Carter, R. A.: Coccidioidal Granuloma: Roentgen Diagnosis, Am. J. Roentgenol. **25**:715-738 (June) 1931; (*b*) Infectious Granulomas of Bones and Joints with Special Reference to Coccidioidal Granuloma, Radiology **23**:1-16 (July) 1934.

Treatment.—The treatment of coccidioidomycosis is in the main symptomatic. Little is known concerning the factors in the host that determine the course of the disease. The factor of race appears to be of definite significance, dark skinned patients, such as Mexicans, Negroes and Filipinos, showing a decidedly greater tendency to have disseminated infections than white patients.[18] The military implication of this observation has been recognized.[2] Factors such as sex, nutrition, previous health and housing appear to be less significant. Women are less apt to have disseminated infections than men and are more prone to have erythema nodosum.[3a]

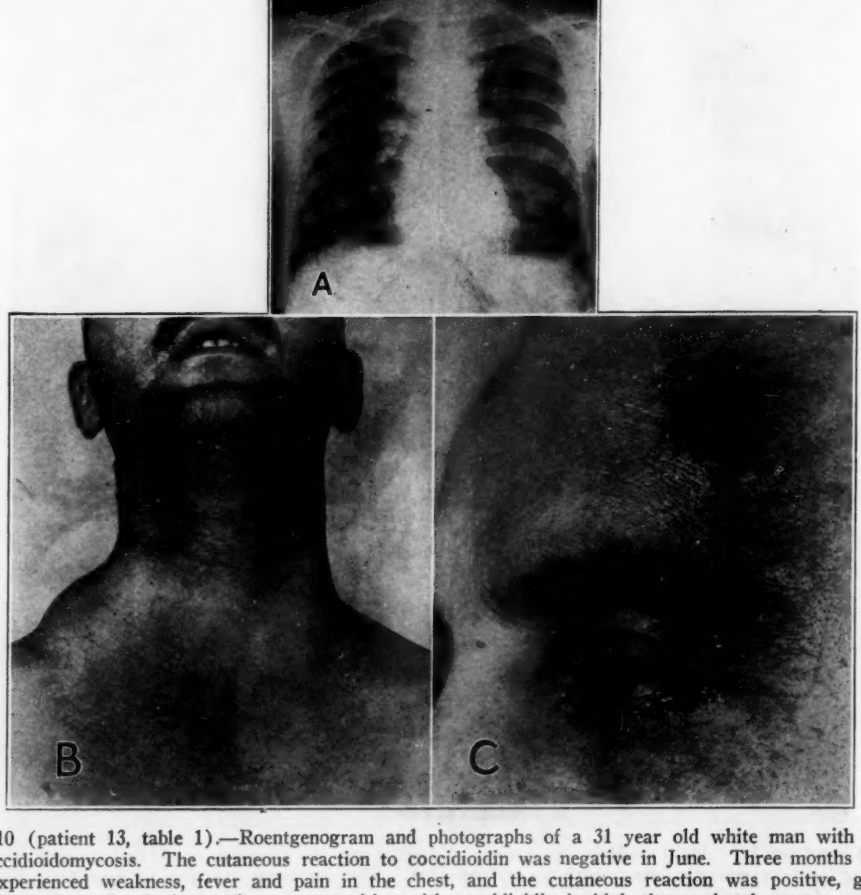

Fig. 10 (patient 13, table 1).—Roentgenogram and photographs of a 31 year old white man with disseminated coccidioidomycosis. The cutaneous reaction to coccidioidin was negative in June. Three months later the patient experienced weakness, fever and pain in the chest, and the cutaneous reaction was positive, gradually becoming negative. Serologic reactions were positive with coccidioidin in high titer. *A*, taken one month after the onset of the illness, shows extensive widening of the superior mediastinum. *B* shows the enlargement of the supraclavicular glands. These glands were aspirated, and the fluid yielded C. immitis on culture and inoculation of animals. *C* shows papillomatous lesion on the left side of the forehead.

Nothing is known concerning the effect of the size of the dose of the infective agent.

The prime objective of treatment is to aid the patient in focalizing the infection. The aid of major importance in accomplishing this end, from a practical standpoint, is rest. From our observations it is felt that the patient should be treated with complete rest in bed, similar to that employed for tuberculous patients, until the temperature is normal and until the sedimentation rate and serologic reactions to coccidioidin indicate that the infection has focalized and that the danger of dissemination has passed. This is advisable even though prolonged hospitalization may be needed in individual cases.

The treatment of patients with pulmonary cavities is a moot question. These patients are evidently immune to generalized dissemination, and there is no danger, such as exists in tuber-

18. (*a*) Coccidioidomycosis Control Program for the Army Air Forces Western Flying Command.[2] (*b*) Peers, R. A.; Holman, E. F., and Smith, C. E.: Pulmonary Coccidioidal Disease, Am. Rev. Tuberc. **45**: 723-740 (June) 1942.

culosis, of "seeding" of the pulmonary tissue from the reservoir of fungus in the cavity. With complete rest in bed some of the cavities may spontaneously close. There is some difference of opinion as to whether or not active treatment should be used even when the cavities persist, inasmuch as these patients suffer no ill effect save that in a few of them hemoptysis may develop occasionally.[19] We employed rest in bed for all of our 5 patients with pulmonary cavities. In addition, we placed a cloth bag containing lead shot over the anterior aspect of the portion of the chest involved. The weight was started at 3 pounds (1.5 Kg.) and gradually increased to 8 pounds (3.6 Kg.). This treatment was first suggested and brought to our attention by a member of the hospital committee on coccidioidomycosis, Major W. Hoyt. It is our impression that the cavities may close more rapidly when treated by this method than with rest in bed alone, though we have not followed a sufficient number of cases to be certain of its value. Because our patients with cavities were clinically well and asymptomatic, we were reluctant to use more than conservative measures. For that reason we have not used pneumothorax in a single case, feeling that the procedure of itself may be followed by complications and that the possibility of restoring a man to military service after pneumothorax is minimal. We employed phrenicolysis in 2 patients. One of the patients (patient 2, table 1) had a cavity in the lower lobe of the left lung that had been stationary for five months previous to the operation. After phrenicolysis the cavity disappeared in three weeks, and the patient was returned to duty (fig. 3). In the other patient (patient 3, table 1) a cavity which measured 3 by 4 cm. was reduced to 2 cm. in diameter by treatment with rest in bed with a bag of lead shot on the chest. Phrenicolysis was done seven months after the cavity was first discovered and was followed by slight decrease in the size of the cavity (fig. 4). This patient has also been returned to duty; he had no complaints and was a skilled noncommissioned officer in an administrative capacity. Another man (patient 7, table 1) presented a small cavity which disappeared eight months after it was discovered, though a small fibrotic density is still present at the site of the cavity. The cavity had remained stationary for five months, after which the patient was treated with the bag of lead shot for three months. This patient also has been discharged to duty. One patient with a cavity was discharged from service because of overage without any particular treatment. The remaining patient with a cavity (patient 8, table 1) had a falling, though still rapid sedimentation rate seven months after the cavity was discovered. He had been treated with the lead shot weight for the past three months, and there has been appreciable decrease in the size of the cavity.

The patients with disseminated coccidioidomycosis offer a most difficult problem. Thymol, iodide, fungus vaccine and roentgen therapy, used in the past, have been disappointing, and the more recent therapeutic agents, sulfonamide compounds and penicillin, have been employed without beneficial effect.[20] The mortality rate for this type of infection has been placed by one observer [3a] at greater than 50 per cent, and others [2] state that after dissemination occurs there is little chance for recovery. The disease may be fulminating, ending fatally in a few weeks, or it may last for months. Fortunately the incidence of dissemination is rare, this form occurring once in every 500 to 1,000 cases of the disease.

In 1 of our patients (patient 11, table 1), an abscess over the sternum was incised, and discharged a diffuse sanguinopurulent drainage for four weeks. With sulfadiazine administered locally and by mouth there was a sharp decrease in the discharge and the sinus closed almost completely within six weeks. We believe that the chemotherapy was of value in combating the secondary infection, though it probably had no effect on the coccidioidomycosis. At the time of discharge to a veterans' facility because of dementia precox this patient had had a normal temperature for one month and had a falling sedimentation rate and coccidioidin antibody titer, and therefore it appeared that he would probably recover. Another patient with disseminated coccidioidal infection (patient 12, table 1) has a destructive lesion of the tenth and eleventh thoracic vertebrae. This patient has had a normal temperature for five months and at the time of this report has a normal sedimentation rate and a decided decrease in the titer of the coccidioidin antibodies. From these facts it is almost certain that his infection is focalizing. Another patient with disseminated infection (patient 13) apparently has a fulminating disease. He had a septic fever, massive suppurative supraclavicular

19. (*a*) Smith.[3a] (*b*) Winn.[15] (*c*) Coccidioidal Granuloma, Special Bulletin no. 57, California State Department of Public Health, 1931.

20. Since this report was submitted, we have studied the effect of two of the acridine dyes, acriflavine hydrochloride and quinacrine hydrochloride, on coccidioidal infection. Because these drugs, particularly acriflavine, appeared to exert an inhibitory effect on C. immitis in vitro in concentrations approximating those which could be expected in vivo after ordinary doses, we tried both of the drugs on 6 patients. Three of these had primary coccidioidal infection, and 3 had disseminated coccidioidal infection. In none of them was there any apparent beneficial effect following administration of these drugs in maximum therapeutic dosages over a period of one month.

glands, enlarged mediastinal glands and a papillomatous lesion on the forehead (fig. 9). Sulfathiazole and sulfadiazine were administered without effect. Immunotransfusions [1d] from donors with focalized coccidioidal lesions were planned for this patient. We were able to secure readily only 1 immune donor with compatible blood; hence the patient was treated with penicillin. Penicillin therapy was started two months after the onset of the infection, at which time the patient's temperature rose daily to a peak of 101 to 102.8 F. During two weeks he was given 2,350,000 Oxford units of penicillin, mainly intramuscularly, though some was given intravenously and a small amount was injected locally into the suppurative supraclavicular glands. During the two weeks of active therapy there was no apparent benefit from the drug. However, almost immediately following the cessation of treatment the temperature dropped and for the past month has reached a peak of 98.8 to 99.4 F. daily. The patient is clinically much improved; he has gained 5 pounds, and there has been a slight drop in the sedimentation rate, though the titer for antibodies of coccidioidin remains high. The papillomatous lesion has disappeared, but the supraclavicular glands are still suppurative, with redness in the overlying skin. It is of importance that cultures of fluid obtained from the suppurative lesion by aspiration on three occasions showed Coccidioides in all mediums and did not reveal the presence of other organisms. This precludes the possibility that the beneficial effect was due to inhibition or destruction of secondary invaders. It is also of interest that broth containing 100 units of penicillin did not inhibit the growth of the coccidioidal fungus from this patient's abscess prior to the institution of treatment.

The remaining patient with disseminated infection also had papillomatous lesions in the skin similar to those of the previous patient. Organisms from one of these lesions were identified as C. immitis. In this patient the infection rapidly became focalized, and now the temperature is normal, the sedimentation rate and the titer for antibodies of coccidioidin are dropping, and the patient is asymptomatic.

It is apparent from the foregoing résumé that the treatment of coccidioidomycosis is in the main symptomatic with the emphasis on rest in bed to aid in preventing dissemination of the infection. Despite this treatment the course may often be prolonged. For patients with primary coccidioidal cavities it would appear that conservative treatment is advisable for a long period and that more radical measures need not be considered unless some complication, such as severe hemorrhage, supervenes. For patients with disseminated infection any treatment which offers some hope of success is indicated, often as a life-saving measure. The use of immunotransfusions, as recently suggested,[1d] is worthy of further trial. The use of penicillin may also be considered in cases of severe involvement in which it appears that the course of the disease is progressively downhill and in which other therapy has been of no avail. Evaluation of treatment cannot be deduced from the few cases in this report; further studies are needed to indicate the worth of the procedures herein noted.

SUMMARY

Attention has been directed to the bizarre clinical picture of chronic coccidioidomycosis and to its importance in military medicine. This report, based on observation of 44 patients, presents the results of our experience with the chronic form of this disease. Fourteen of these patients were shown to have active coccidioidal infection, 10 having infections of the primary benign type and 4 having infections of the progressive disseminated type. Thirty other patients were suspected of having coccidioidomycosis because of the presence of a positive cutaneous reaction to coccidioidin, but this was subsequently not confirmed. A careful clinical study of these 44 patients has indicated the relative clinical value and the limitations of the recognized diagnostic procedures, including investigation of the patient's history, physical examination, cutaneous and serologic tests with coccidioidin, culture and animal inoculation for C. immitis, biopsy, blood counts, determination of the sedimentation rate and roentgen examination, in aiding to distinguish patients with active coccidioidomycosis from those with conditions simulating coccidioidal infection. The case reports herein cited illustrate the common diagnostic errors and pitfalls that may be encountered in the clinical study of patients suspected of having coccidioidal infection.

Our observations indicate that medicinal treatment usually has little effect on this disease and that the primary consideration of therapy is rest in bed to aid focalization of the infection. Immobilization of the lung by a lead shot bag on the chest, which was used on some patients in this series, is a procedure not previously utilized, to our knowledge, and appeared to be of some benefit in helping to close the thin-walled cavities caused by the coccidioidal infection. Acridine dyes (acriflavine hydrochloride and quinacrine hydrochloride), the use of which has not previously been reported in this disease, were tried but proved of no value.

From a military standpoint, even though these patients with chronic coccidioidal infection may require long periods of hospitalization, they may often be returned to useful military service.

NATURE AND TIME ACTION OF MODIFICATIONS OF PROTAMINE ZINC INSULIN

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EVANSTON, ILL.

Since insulin must be used parenterally and since injections once daily seem to be the inevitable minimum because of normal eating and sleeping habits, it should be possible to select an ideal insulin for routine treatment of diabetes mellitus.

Protamine zinc insulin is too slow and prolonged in action for best control of severe diabetes. When large doses are used, insulin shock is prone to occur during fasting, especially at night.[1] In severe diabetes heavy glycosuria occurs after meals.[2] Supplementary injection of ordinary insulin helps to correct such faults, but even so control in many cases remains imperfect and the penalty of multiple injections is imposed.[3]

Mild diabetes is easily controlled with protamine zinc insulin injected once daily.[4] This does not imply that it is an ideal insulin, because almost any type of depot insulin will do just as well in small doses for mild diabetes. Even though glycosuria may not follow meals, postprandial hyperglycemia does occur, which indicates the same inadequacy of protamine zinc insulin as in severe diabetes.

These factors and others have been reviewed at length by Colwell, Izzo and Stryker.[5] They are recognized by all experienced in the treatment of diabetes and require no elaboration. Because of them interest in improved insulins has grown rapidly during the last several years that protamine zinc insulin has been in use. Timing studies and therapeutic trials with various modifications have been reported, with improved control as a general rule.[6] Yet no uniformity of technic has developed, and some misunderstanding regarding the nature and action of certain modifications is apparent. The work here reported was undertaken in the hope of clarifying and helping to standardize therapeutic technic with improved insulins.

In the field of growing usefulness of mixtures of regular insulin and protamine zinc insulin made from commercial supplies now available, there is general agreement on three major points. First, mixtures containing suitable excesses of insulin show increased prompt and lessened delayed action. Second, "suitable excesses" have been found to contain at least as much ordinary insulin as protamine zinc insulin in the mixture, usually more, and in some cases as much as five times the protamine zinc insulin used. Third, such mixtures control severe diabetes better than protamine zinc insulin and avoid multiple daily injections.

What is not yet clear to most users of mixtures are the answers to the following questions concerning them:

1. In what form is the insulin in them as modified by admixture, and what is the effect on it of buffering to the p_H of tissue fluids?

From the Department of Medicine, Evanston Hospital and Northwestern University Medical School, aided by a grant from Eli Lilly & Co., Indianapolis.

1. Lawrence, R. D., and Archer, N.: Some Experiments with Protamine Insulinate, Brit. M. J. **1**:747-749 (April 11) 1936. Kepler, E. J.: Clinical Experience with Protamine Zinc Insulin, J. A. M. A. **110**:92-96, (Jan. 8) 1938. Neuhoff, F., and Rabinovitch, S.: Protamine Zinc Insulin, Arch. Int. Med. **62**:447-460 (Sept.) 1938.

2. Campbell, W. R.; Fletcher, A. A., and Kerr, R. B.: Protamine Insulin in the Treatment of Diabetes Mellitus, Am. J. M. Sc. **192**: 589-600 (Nov.) 1936. Joslin, E. P.: Protamine Insulin, J. A. M. A. **109**: 497-503 (Aug. 14) 1937.

3. Joslin, E. P.; Root, H. F.; Marble, A.; White, P.; Joslin, A. P., and Lynch, G. W.: Protamine Insulin, New England J. Med. **214**:1079-1085 (May 28) 1936. Mosenthal, H. O.: Protamine Zinc Insulin: Clinical Application, J. A. M. A. **110**:87-90 (Jan. 8) 1938.

4. Joslin, E. P.: Protamine Insulin and Its Advantages, New England J. Med. **215**:1166-1168 (Dec. 17) 1936.

5. Colwell, A. R.; Izzo, J. L., and Stryker, W. A.: Intermediate Action of Mixtures of Soluble Insulin and Protamine Zinc Insulin, Arch. Int. Med. **69**:931-951 (June) 1942.

6. (*a*) Sparks, M. L., and John, H. J.: The Clinical Use of Mixtures of Insulins, Ohio State M. J. **39**: 226-228 (March) 1943. (*b*) Olmstead, W. H.: Observations on the Treatment of Diabetes Mellitus, J. Iowa M. Soc. **33**:95-101 (March) 1943. (*c*) Hildebrand, A. G., and Rynearson, E. J.: Clinical Experience with Mixtures of Protamine Zinc and Unmodified Insulins, Arch. Int. Med. **72**:37-45 (July) 1943. (*d*) Peck, F. B.: Treatment of Uncomplicated Diabetes with Mixtures of Insulin and Protamine Zinc Insulin, J. Indiana M. A. **36**:340-348 (July) 1943. (*e*) MacBryde, C. M., and Roberts, H. K.: Modified Protamine Zinc Insulin: An Improvement on Standard Protamine Zinc Insulin, J. A. M. A. **122**:1225-1231 (Aug. 28) 1943. (*f*) Colwell, A. R., and Izzo, J. L.: Protamine Zinc Insulin Modified for Accelerated Action, ibid. **122**:1231-1236 (Aug. 28) 1943.

2. Must mixtures be individualized to the needs of different patients (extemporaneous mixtures [Peck [7]]), or may a fixed modification be selected and marketed?

3. Is any other modification, such as globin zinc insulin, preferable to the most efficient protamine zinc insulin available?

The following data provide insight into the first of these problems and add to accumulating experience concerning the last two.

UNBUFFERED MIXTURES OF INSULIN AND PROTAMINE ZINC INSULIN

When crystalline insulin (solution of zinc insulin crystals) is added to protamine zinc insulin in increasing amounts, physical and chemical changes occur which correspond with variations in the time activity of the mixtures on injection. These changes are shown in table 1 and chart 1 for U-80 insulins of one manufacturer.[8] No mixture containing less insulin than protamine zinc insulin is included, because the changes are inconsequential.

The whole mixture naturally increases in acidity with added insulin, because protamine zinc insulin is buffered to p_H about 7.2 and the p_H of the crystalline insulin added is 2.8 to 3.0. As more and more of the acid insulin solution is added to the buffered protamine zinc insulin suspension, the p_H decreases slowly. There is no visible change in the gross character of the mixtures up to the addition of 6 parts insulin (6:1) with p_H 4.4. With more insulin and lower p_H values the amount of suspended sediment diminishes rapidly until at the p_H (4.0) of a 10:1 mixture it disappears completely, all insoluble fractions going into solution at ordinary temperatures. Since protamine zinc insulin becomes soluble at this p_H, there is no reason to believe that the added insulin causes its solution by any means other than its acidifying action. "Acid," or "clear," protamine zinc insulin below this p_H is well known.[9]

TABLE. 1.—*Soluble and Insoluble Insulin Fractions and p_H in 80 Units of Unbuffered Mixtures of Crystalline Insulin and Protamine Zinc Insulin from 1:1 to 11:1 Proportions (Unmixed Protamine Zinc Insulin* and Crystalline Insulin† Included for Comparison)*

Proportions Cryst. : P. Z. I.	p_H of Mixture	Insulin in Supernatant, Units (in 80 units of mixture)	Volume of Precipitate, Cu. Mm.
0* : 1	7.27	2.4	53
1 : 1	6.76	1.6	52
2 : 1	6.21	0.9	49
3 : 1	5.82	2.0	45
4 : 1	5.22	3.2	31
5 : 1	4.76	6.4	30
6 : 1	4.37	Colloidal	26
8 : 1	4.02	Colloidal	1
10 : 1	3.95	80	0
11 : 1	3.76	80	0
1† : 0	3.00	80	0

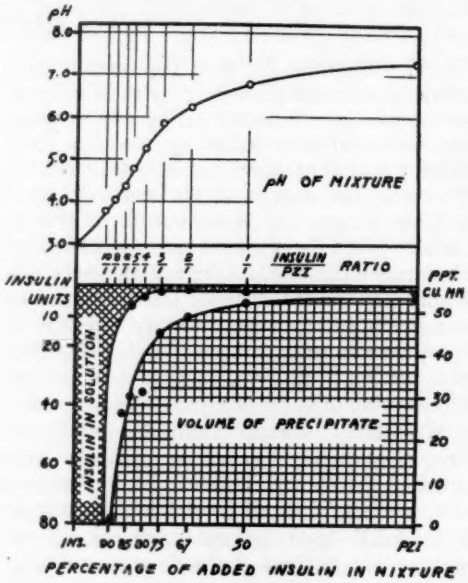

Chart 1.—Soluble and precipitated insulin components and p_H of unbuffered mixtures of crystalline insulin and protamine zinc insulin. Until the insulin used in the mixture exceeds 3:1 proportions and the p_H falls below 5.8, there is little change in the resultant soluble and insoluble fractions. Complete solution occurs in 10:1 proportions at p_H about 4. Intermediate mixtures at p_H 4.4 to 4.0 show colloidal precipitation. The two commercial insulins used in the mixtures are shown at the extremes for comparison. (Data taken from table 1.)

Therapeutic usefulness has been reported to lie within the zone of the 1:1 to 5:1 mixtures by all recent observers.[6] The modified action of two such mixtures (2:1 and 3:1) is reproduced from the former report [5] in chart 2 for reference. They are clearly intermediate in promptness, intensity and duration of action between ordinary insulin and protamine zinc insulin. These time action curves do not show, however, whether the accelerated effect is due to excess insulin in so-

7. Peck, F. B.: Approximate Insulin Content of Extemporaneous Mixtures of Insulin and Protamine Zinc Insulin, Ann. Int. Med. **18**: 177-181 (Feb.) 1943.

8. Supplied by Eli Lilly & Co., Indianapolis.

9. (*a*) Warvel, J. H.: Protamine and Other Slow Acting Insulins and Their Clinical Application: Review of Medical Progress, Ohio State University College of Medicine, 1940, p. 140. (*b*) Bailey, C. C., and Marble, A.: Histone Zinc Insulin, Globin (Zinc) Insulin, and Clear Protamine Zinc Insulin: A Comparative Study of Their Action, J. A. M. A. **118**:683-690 (Feb. 28) 1942.

lution, in uncombined form in the precipitate or in combination with protamine and zinc in the precipitate. Inasmuch as the action is clearly monophasic, it suggests the existence in each mixture of a single protamine zinc insulin complex which releases insulin more rapidly after injection than ordinary protamine zinc insulin rather than a mixture of two insulins with widely varying action properties.

Thorough centrifuging of 1:1 to 5:1 mixtures separates them into soluble and insoluble insulin fractions (appendix I), which were measured by approximate methods. These methods are described in detail in appendixes II and III. Volumes of precipitate were measured by means of the familiar hematocrit technic. Supernatant

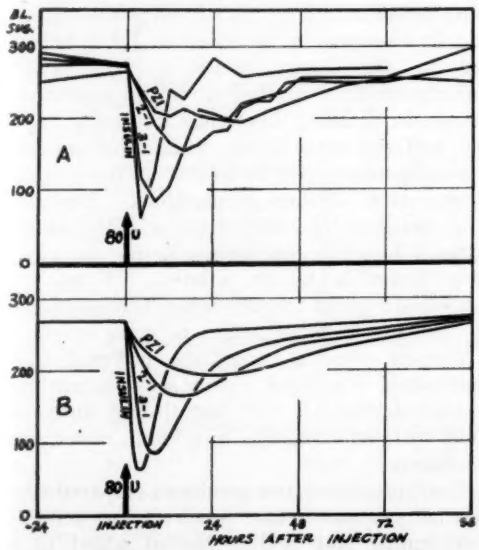

Chart 2.—Average (*A*) and smoothed (*B*) blood sugar curves showing the time action of 80 unit doses of regular insulin, protamine zinc insulin and two mixtures containing excesses of insulin. The observations were made on stabilized diabetic patients with constant hyperglycemia induced by four hour meals of approximately equal value, as described in appendix IV.

insulin in solution was measured by means of a colorimetric method for cystine adapted to insulin. This depends on reduction of cystine to cysteine by sodium cyanide, prevention of precipitation with urea and appearance of a clear blue color on standing with a phosphotungstic acid reagent. Known crystalline insulin solutions were used as standards. This method has been proved quantitatively satisfactory within 10 per cent limits of error when insulin is kept in solution and when the material analyzed contains no other source of cystine than insulin.

As demonstrated in table 1 and chart 1, even when insulin is added in excess over protamine zinc insulin up to 4:1 proportions (up to 80 per cent soluble insulin) little of it remains in soluble form in the supernatant. In other words, even when as much as 64 units of soluble insulin is mixed with 16 units of commercial protamine zinc insulin, only about 3 units, or $\frac{1}{20}$ of the added insulin, remains in solution. Ninety-five per cent of the insulin added to one fourth as much protamine zinc insulin is precipitated. Thus the accelerated and more intense action of such mixtures cannot be due to ordinary insulin in solution. These values are somewhat at variance with those reported by Peck,[10] but he pointed out that his figures were only approximate.

This surprising fact has received previous comment but no satisfactory explanation. It has been assumed that the excess insulin is precipitated as ordinary protamine zinc insulin by the excess protamine present in protamine zinc insulin.[11] Yet if this were true such mixtures should be identical in action with ordinary protamine zinc insulin, and they are not, as the curves in chart 2 illustrate. Furthermore, no "free" protamine can be demonstrated in the supernatant from ordinary protamine zinc insulin.

With more acid mixtures, containing greater excesses of insulin (6:1 and beyond), a colloidal precipitate appears at p_H about 4.4, which makes it impossible to separate and study the soluble and insoluble fractions. At p_H 4.0 in 10:1 proportions all fractions go into solution ("acid," or "clear," protamine zinc insulin [9]).

Precipitation of practically all insulin added in 1:1 to 4:1 mixtures is further proved by estimations of the amounts of precipitate in them. After thorough centrifuging, their volumes of precipitate remain fairly constant in the range of that of ordinary protamine zinc insulin, as shown in table 1 and chart 1. Since these are volumetric rather than gravimetric estimations, they probably vary from quantitative data by virtue of differences in the size of crystals and their compactness in the sediment. Yet their constancy indicates continued precipitation of added insulin as the proportion of protamine zinc insulin decreases. Thus, the absence of added soluble insulin in the supernatants is confirmed by finding it in the precipitates. Ulrich noted this constancy measured by similar methods.[11a]

There are three possible explanations for this phenomenon: Offhand it would seem that excess protamine in solution in protamine zinc insulin must precipitate added insulin as ordinary pro-

10. Peck, F. B.: Action of Insulins, Proc. Am. Diabetes A. **2**:69-83, 1942.

11. (*a*) Ulrich, H.: Clinical Experiments with Mixtures of Standard and Protamine Zinc Insulins, Ann. Int. Med. **14**:1166-1179 (Jan.) 1941. (*b*) Peck.[10]

tamine zinc insulin. That this is not the case is proved by the fact that the supernatant from ordinary protamine zinc insulin does not precipitate insulin added to it.

The second possible explanation was suggested by Ulrich.[11a] Insulin itself precipitates inside the p_H range of 5.0 to 6.0. Its isoelectric point is 5.3 to 5.35.[12] Since the most popular mixtures lie within this p_H range, the added insulin might precipitate as such without excess protamine, resulting in precipitates which are mixtures of crystals of insulin and protamine zinc insulin. Yet a 1:1 mixture with p_H outside the zone in which insulin is insoluble shows no more supernatant insulin than protamine zinc insulin itself. Furthermore, buffering studies (reported in the next section) show clearly that this could not be the correct explanation.

The third possible interpretation conceives of an entire series of protamine zinc insulins, of which the commercial product is only one, in which increasing amounts of insulin may be combined in an increasingly "insulin-saturated" insoluble complex up to a saturation limit. Peck has hinted at this interpretation,[7] which on analysis has proved to be correct.

STUDY OF BUFFERED MODIFICATIONS

If mixtures of regular and protamine zinc insulin were composed of simple combinations of rapid-acting and slow-acting insulins in various proportions, as is generally assumed, it should be possible to separate them by buffering. Insulin is easily soluble at p_H 7.0 to 7.5, but protamine zinc insulin is most insoluble at p_H 7.2, by deliberate selection of the protamine used.[13] Acid mixtures rebuffered to p_H 7.2, then, should release their excess insulin into the supernatant. The modification used and described by MacBryde [6e] is prepared in this way and judged to be a mixture of rapid-acting insulin in solution and protamine zinc insulin in suspension. As the following experiments show, this may be true under given conditions of mixing, but there are many variables, including amount of excess insulin, p_H, time of contact before buffering, temperature, amount of zinc present and probably stability of proportions even after buffering.

To illustrate some of these factors the estimations of supernatant insulin shown in chart 3 were made. In this series a single mixture of proved therapeutic usefulness (2:1), containing 2 parts of U-80 crystalline insulin mixed with 1 part of U-80 protamine zinc insulin (p_H 6.2),[8] was buffered with dibasic sodium acid phosphate to p_H 7.0 to 7.5. Its supernatant insulin in solution was then separated by centrifuging and decanting and measured roughly by injection into a diabetic patient with a stabilized blood sugar level (appendix IV). Supplementary semiquantitative chemical estimations for insulin were also performed on the supernatant fraction. This method is described in detail in appendix V. It has been found reliable as a quick approximate method for measuring cystine, and therefore insulin, when no other source of sulfur is present in the unknown. Insulin in solution, as estimated by this method, is shown by the figure superimposed on each blood sugar curve in chart 3.

As demonstrated in chart 3, even when the p_H of a 2:1 mixture is increased to 7.2, relatively little of the excess insulin appears in the supernatant if the insulins are mixed some hours before rebuffering. When they are freshly mixed and buffered immediately, measurable amounts of insulin may appear in soluble form, especially in the more alkaline preparations. However, even then less than half of the added insulin is released into the supernatant under conditions most favorable to its solution. When the insulins are mixed for days before being buffered, no measurable blood sugar–reducing effect is seen even when 2 cc. of the buffered U-80 supernatant is injected. More sensitive methods of analysis indicate that less than 5 units per cubic centimeter remains in solution under such conditions.

Thus, protamine zinc insulin as prepared commercially appears to have a powerful capacity for precipitating and binding insulin added to it. This capacity is stronger in slightly acid suspension mediums but is still apparent even when the mixture is buffered to p_H 7.2 or higher. Added insulin may not precipitate as completely if buffered immediately. If precipitation is permitted to take place in slightly acid mediums before the buffering, little added insulin (up to twice the amount present in protamine zinc insulin) is released on buffering. Other studies, not reported here, indicate that other variables, such as zinc and temperature, may affect the proportion of soluble insulin present in buffered mixtures. There is also good reason to believe that even buffered mixtures which do contain insulin in solution originally may lose it by precipitation on standing in the cold a long time.

These estimations of supernatant soluble insulin demonstrate the futility of planning modifications containing fixed amounts of insulin in solution. Unless exacting conditions of mixing

12. Wintersteiner, O., and Abramson, H. A.: The Isoelectric Point of Insulin: Electrical Properties of Adsorbed and Crystalline Insulin, J. Biol. Chem. **99**: 741-753, 1933.

13. Hagedorn, H. C.; Jensen, B. N.; Krarup, N. B., and Wodstrup, I.: Protamine Insulinate, J. A. M. A. **106**:177-180 (Jan. 18) 1936.

and buffering are carried out, the component of soluble insulin in any mixture, and thus its action, may be variable. Further, there would seem to be no reason to fear that acid mixtures buffered by tissue fluids on injection might suddenly release dangerously large amounts of rapid-acting insulin.[6e] In fact, such mixtures, even when injected immediately after mixing, have been shown by experiment and repeated clinical trial to be identical in action with premixed and buffered preparations of the same type.

From the foregoing experiments and discussion it seems apparent that protamine zinc insulin mixed with excesses of soluble insulin forms a series of precipitates with the following properties:

1. More prompt and intense and less prolonged activity than commercial protamine zinc insulin.
2. Monophasic action on injection, suggesting insulin in a single complex rather than in two (rapid-acting and slow-acting) compounds.
3. Little insulin in solution, even when added in 4:1 excess, or in 2:1 excess even when buffered to p_H 7.2.
4. The amount of suspended insoluble insulin fairly constant even when the proportion of protamine to zinc is reduced to about one third of that in commercial protamine zinc insulin.
5. The excess insulin combined firmly in a protamine zinc insulin complex, because it does not dissolve on buffering to p_H 7.2 unless buffered immediately after addition.

Therefore it may be assumed that protamine in the presence of zinc has an elastic capacity for combining insulin in a more or less saturated insoluble complex the sugar-reducing time activity of which depends on its degree of saturation with insulin. This capacity for saturation lies within the range of mixtures proved to be useful therapeutically. The most important variable in this series of protamine zinc insulin complexes appears to be the proportion of protamine and zinc to insulin in the combination.

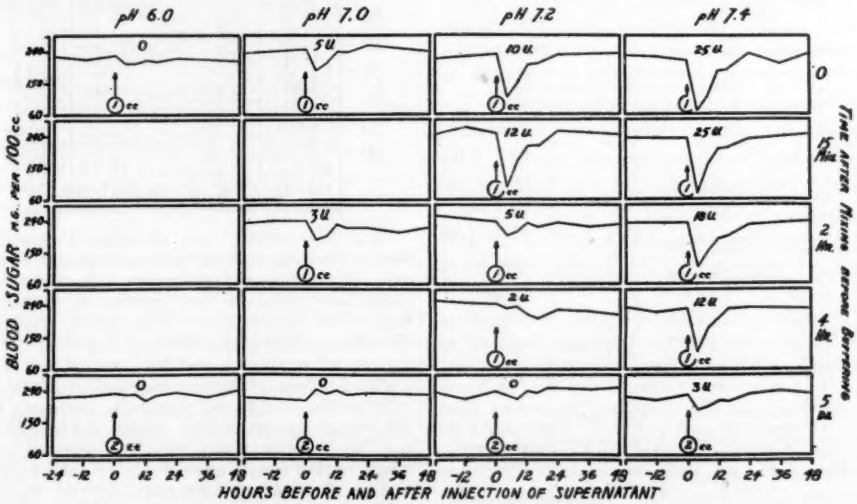

Chart 3.—The blood sugar–reducing effect of 1 to 2 cc. doses of supernatant from a U-80 2:1 mixture under various conditions of buffering. The effect of increasing alkalinity is shown from left to right. The effect of immediate and delayed buffering is shown from above to below. The dosage of supernatant after delayed buffering (below) was doubled. The blood sugar curves were made on a stabilized diabetic patient with constant hyperglycemia. The figures superimposed on each curve show the insulin in each supernatant as estimated by precipitation of lead sulfide after hydrolysis (appendix V).

CAPACITY OF PROTAMINE FOR COMBINING INSULIN

In order to test the validity of the foregoing assumptions, an analysis was made of a series of protamine zinc insulin complexes in which the only variables were the amounts of protamine and zinc, which varied with each other in the same relation. These contained identical amounts (100 units) of crystalline insulin (solution of zinc insulin crystals, U-80[14]), and they were all buffered to p_H about 7.2. The same protamine as is used commercially (from sperm or testis of the Columbia River salmon, family Salmonidae[15]) was added in increasing amounts

14. New and Nonofficial Remedies, Chicago, American Medical Association, 1944, p. 427.

15. New and Nonofficial Remedies,[14] p. 431. This was supplied by Eli Lilly & Co.

from 0.1 to 1.0 mg. per hundred units of insulin. Zinc was used in about the same ratio to protamine as in standard protamine zinc insulin (20 per cent of the protamine, or 0.02 to 0.2 mg. per hundred units) in all preparations. Additions were made uniformly in the order of (1) insulin, (2) protamine in 0.04 per cent aqueous solution, (3) zinc and (4) buffer in the form of 2 per cent dibasic sodium acid phosphate to p_H about 7.2. Room temperatures existed throughout. The methods used in the estimations of soluble and insoluble insulin fractions are described in appendix II and III.

Table 2 shows the ingredients of these preparations and the amounts of their soluble and insoluble insulin components. In chart 4 their supernatant insulin content and volumes of insoluble protamine zinc insulin precipitate obtained by centrifuging are plotted in relation to ordinary insulin and protamine zinc insulin.

Crystalline insulin, containing no protamine, has all of its insulin in solution, of course. The addition of 0.1 mg. of protamine and 0.02 mg. of zinc, with the p_H adjusted to about 7.2, apparently precipitated about one fourth of the 100 units used, as indicated by the supernatant fraction of 76 units and the precipitated volume of about one-fourth that present in protamine zinc insulin. With increasing additions of protamine up to 0.4 mg. and zinc in proportion, the supernatant insulin decreased and the precipitated fraction increased rapidly. From 0.4 to 1.0 mg. of protamine per hundred units at p_H 7.2, there was little change in the soluble and insoluble fractions. Each 100 units of insulin was almost completely precipitated. The precipitates were fairly constant in volume and equal to that of standard protamine zinc insulin, and the supernatant contained only 2.5 to 7 per cent of the insulin used originally.

These data show fair agreement between the amount of insulin precipitated with protamine and zinc and the difference between the quantity of insulin used and that recovered in the supernatant. Thus, each unit of insulin that disappeared from solution was seen as about 1 cu. mm. of precipitate when centrifuged. The rate of precipitation was about 25 units, or 1 mg., of insulin for each 0.1 mg. of protamine added until most of the 100 units used in these preparations was precipitated with about 0.5 mg. of protamine. Thereafter further additions of protamine and zinc did not affect the amounts of soluble insulin, and the insoluble fraction increased only a little. Presumably the protamine and zinc enter the precipitate, as shown by the absence of demonstrable protamine in the supernatant and a change in the time activity of the precipitate, reported in the next section. They may account for the slight increase in volume of the precipitate after precipitation of insulin ceases.

The simplest possible expression of these relationships is obtained by recalculation of the data

TABLE 2.—*Soluble and Insoluble Insulin Fractions in Protamine Zinc Insulin Modifications Buffered to About p_H 7.2, All Made from 100 Units of Crystalline Insulin but Containing Protamine and Zinc in Variable Amounts Less than Standard Protamine Zinc Insulin (Crystalline Insulin* and Standard Protamine Zinc Insulin† Included for Comparison)*

Ingredients				Soluble and Insoluble Insulin Fractions	
Insulin, Units	Protamine, Mg.	Zinc, Mg.	p_H	Insulin in Supernatant, Units	Volume of Precipitate, Cu. Mm.
100*	0	0.02	3.0	100	0
100	0.1	0.02	7.23	76	26
100	0.2	0.04	7.25	37	56
100	0.3	0.06	7.20	19	81
100	0.4	0.08	7.19	7.1	91
100	0.5	0.10	7.17	5.0	97
100	0.6	0.12	7.18	3.8	105
100	0.7	0.14	7.16	2.9	99
100	0.8	0.16	7.42	3.4	102
100	0.9	0.18	7.21	2.5	100
100	1.0	0.20	7.18	3.5	104
100†	0.75 to 1.25	0.20	7.27	3.0	101

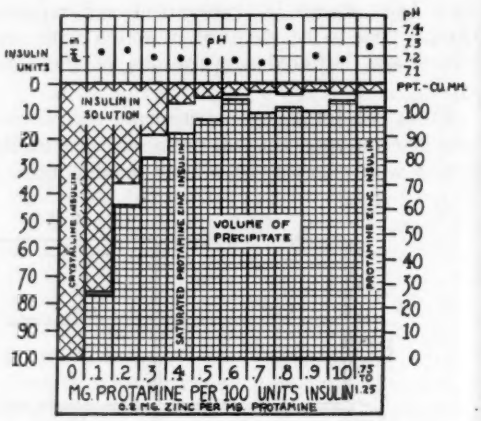

Chart 4.—Soluble and precipitated components of insulin resulting from increasing additions of protamine and zinc to 100 units of insulin at p_H about 7.2. Crystalline and protamine zinc insulin are included at extremes for comparison. (Data taken from table 2.) Almost complete precipitation of 100 units occurs when 0.4 mg. of protamine and 0.08 mg. of zinc are added. Up to this level protamine must be considered saturated with insulin. Additional protamine above this level does not change the amounts of soluble and insoluble insulin, but the excess protamine forms unsaturated precipitates which release insulin more slowly on subcutaneous injection, as shown in charts 6 and 7.

in terms of 1 mg. of protamine and 0.2 mg. of zinc. By such calculation the fate of insulin added in increasing amounts to 1 mg. of protamine is seen. This is the reverse of the sequence just described, in which protamine was added in increasing amounts to 100 units of insulin. Table 3 and chart 5 show the results of the data so recalculated.

TABLE 3.—*Recalculation of Data from Table 2 Showing Soluble and Insoluble Insulin Fractions per Milligram of Protamine and 0.2 Mg. of Zinc (Crystalline Insulin* and Protamine Zinc Insulin† Included for Comparison)*

Protamine, Mg.	Zinc, Mg.	Insulin, Units	p_H	Insulin in Supernatant, Units	Volume of Precipitate, Cu. Mm.
0.75 to 1.25*	0.2	100	7.27	3.0	101
1.0	0.2	100	7.18	3.5	104
1.0	0.2	111	7.21	2.8	111
1.0	0.2	125	7.42	4.3	128
1.0	0.2	143	7.16	4.1	142
1.0	0.2	167	7.18	6.3	175
1.0	0.2	200	7.17	10	194
1.0	0.2	250	7.19	18	228
1.0	0.2	333	7.20	63	267
1.0	0.2	500	7.25	185	280
1.0	0.2	1,000	7.23	760	260
0†	0.2	1,000	3.0	1,000	0

One milligram of protamine appears to be capable of precipitating about 250 units, or 10 mg. of insulin at p_H 7.2. Above this ratio all insulin added appears in solution.

One milligram of protamine and 0.2 mg. of zinc in solution at p_H 7.2 contain no insulin, of course, either in soluble or in precipitated form. The addition of 100 units of insulin yields a measurable precipitate, which contains 97 per cent of the insulin, since only about 3 units is found in solution in the supernatant. This is approximately the same as protamine zinc insulin as now marketed, containing from 0.75 to 1.25 mg. of protamine and 0.2 mg. of zinc per hundred units at p_H 7.2.[15] Further additions of insulin to 1.0 mg. of protamine with zinc, with the p_H kept at 7.2, increase the volume of precipitate in the same proportion as the insulin added, and little more supernatant insulin is found, until the aforementioned saturated level is reached. Above about 250 units per mg. of protamine (0.4 mg. per hundred units), the supernatant soluble insulin shows a sharp increase and thereafter all added insulin makes its appearance in solution. At about 300 units per milligram of protamine the insoluble fraction also stops increasing with further additions of insulin. As demonstrated by the superimposed curves in chart 5, the two fractions added together account for practically all insulin used, if 1 cu. mm. of precipitate is considered to represent 1 unit of insulin.

Here the relation between insulin and protamine with zinc is clearly evident. At ordinary temperatures and p_H 7.2 practically all insulin up to 250 to 300 units added to 1 mg. of protamine with zinc is precipitated in an insoluble protamine zinc insulin compound. Above this level all added insulin is recovered in the supernatant and the precipitate increases no more. In the preparations containing less than 250 units per milligram of protamine the excess protamine in the precipitate is proved by its absence from the supernatant and by the fact that it can precipitate additional insulin.

Thus the preparations precipitating 250 to 300 units per milligram must be considered saturated with insulin, since all insulin added above this

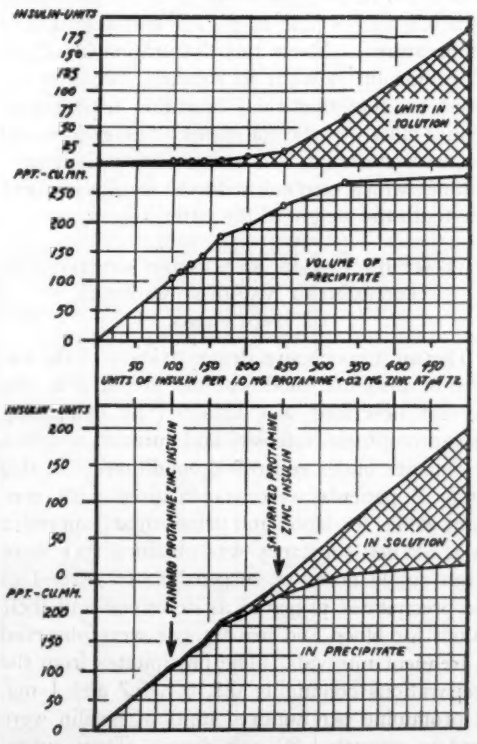

Chart 5.—Effect of increasing additions of insulin to 1 mg. of protamine with 0.2 mg. of zinc at p_H 7.2. These data, taken from table 3, were obtained by recalculating in terms of 1 mg. of protamine the data shown in table 2 and chart 4. Up to 250 to 300 units per milligram of protamine practically all insulin is precipitated in an insoluble compound which becomes more saturated with insulin as more is added to the fixed 1 mg. protamine. Above this level all insulin remains in solution, a saturated compound being left in the precipitate.

level remains in solution. The precipitates in the preparations with excess insulin in solution must also be considered saturated, since no more

insulin joins the precipitate. Accordingly, those preparations containing and precipitating less than 250 units per milligram of protamine must be considered unsaturated with insulin, because they can precipitate more insulin and little remains in solution.

Here again it is clear (table 3) that at p_H 7.2 each unit of insulin used appears as about 1 cu. mm. of precipitate up to about 250 units for 1 mg. of protamine and 0.2 mg. of zinc. Above this level practically all insulin added appears in solution. Below this level the excess of protamine and zinc is not reflected in the amount of precipitated or soluble insulin.

In the simplest possible terms, these findings indicate that 250 to 300 units (10 to 12 mg.) of insulin can be precipitated by 1 mg. of protamine with 0.2 mg. of zinc at p_H 7.2 and at ordinary temperatures. Above this "saturation level" all added insulin remains in solution. It is of interest to note that this saturation level corresponds closely to the statement by Hagedorn and his associates that "the amount of various protamines which combine with the insulin is about one-tenth the weight of the latter." [13]

TIME-ACTION CURVES OF WASHED PRECIPITATES CONTAINING VARIOUS AMOUNTS OF PROTAMINE

The most conclusive demonstration of the nature of insoluble modifications prepared in the manner described was obtained by comparing their promptness, intensity and duration of action in diabetes under controlled conditions. In this study 2 patients were standardized with constant values for blood and urine sugar (appendix IV). When constancy was obtained they were given single doses of suspensions of several of the precipitates prepared as described and their values for blood and urine sugar were observed at frequent intervals. The precipitates from the preparations containing 0.1, 0.4, 0.7 and 1 mg. of protamine per hundred units of insulin were used in estimated 80 unit doses. Their supernatant fractions were discarded, and they were washed at least twice with p_H 7.2 buffer before resuspension and injection. This was done to insure removal of any quickly soluble and hence rapid-acting insulin before testing. The washings were practically free from insulin by chemical test.

Their time-action curves are shown and compared with those resulting from insulin and protamine zinc insulin in chart 6. Chart 7 shows the composite data averaged for both patients and each type of insulin. The 0.1 and 0.4 mg. curves were averaged and are shown as the record for a "saturated" preparation. The 0.7 and 1.0 mg. curves were likewise averaged, as representative of an "unsaturated" preparation. Chart 7 also shows these curves arbitrarily smoothed to eliminate minor variations obviously not due to the action of insulin. The washed precipitates are clearly intermediate in action between insulin and protamine zinc insulin.

Comparison of these curves with those in chart 1 shows beyond doubt that these insoluble protamine zinc insulin complexes containing variable quantities of protamine and zinc, even after thorough washing to remove any easily soluble insulin fraction, exhibit characteristics of promptness, intensity and duration of action which are intermediate between those of insulin and those of protamine zinc insulin, as do simple mixtures. The preparations used in the two series of experiments, containing approximately the same amounts of protamine and zinc (the 2:1 simple mixture and the one with 0.4 mg. of protamine per hundred units of precipitate), exhibit almost identical characteristics of peak action and total duration of effect. Both of these are insulin-saturated precipitates, containing 250 to 300 units per milligram of protamine, the critical level above which insulin remains in solution at p_H 7.2. Thus it may be assumed that the timing and intensity characteristics of mixtures containing less protamine and zinc and more insulin than protamine zinc insulin are determined by their protamine and zinc content rather than by any simple component of soluble insulin.

Further proof of insulin saturation in the complex containing 0.4 mg. of protamine per hundred units of insulin (1 mg. per 250 units) is to be found in a comparison of its action curve with that of the precipitate from the preparation containing only one-fourth as much protamine and zinc (0.1 mg. of protamine per hundred units of insulin). The latter preparation was shown in table 2 and chart 4 to contain about three fourths of its 100 units in the supernatant. This was discarded, an estimated 25 units being left in the washed precipitate. The volume of its precipitate was also approximately one-fourth that of the precipitate from the preparation containing 0.4 mg. of protamine per hundred units and little insulin in the supernatant. On the basis of these estimations, the dose of the 0.1 mg. precipitate was quadrupled for testing by injection. Its curves were identical in intensity and timing with those of the 0.4 mg. precipitate.

Thus it may be presumed that in both precipitates protamine combined with insulin in a firm,

relatively insoluble complex in the ratio of 0.1 mg. of protamine per 25 units of insulin, or 1 mg. per 250 units, or 10 mg. Beyond this saturation limit additional insulin remained in solution at p_H 7.2. It was discarded from the preparation containing only 0.1 mg. of protamine per hundred units, and the smaller volume of precipitate, identical in action but one fourth in amount as compared with the other saturated (0.4 mg.) preparation, had to be quadrupled in order to obtain a comparable sugar-reducing effect.

To sum up, it seems clear that the important factors which govern the rate of action of protamine zinc insulin modifications are the amounts of protamine and zinc present in proportion to insulin. The protamine now used in the manufacture of protamine zinc insulin when supplemented by about 20 per cent as much zinc is capable at p_H 7.2 of precipitating ten to twelve times its weight, or approximately 250 to 300 units of insulin per milligram of protamine, in a relatively insoluble protamine zinc insulin modi-

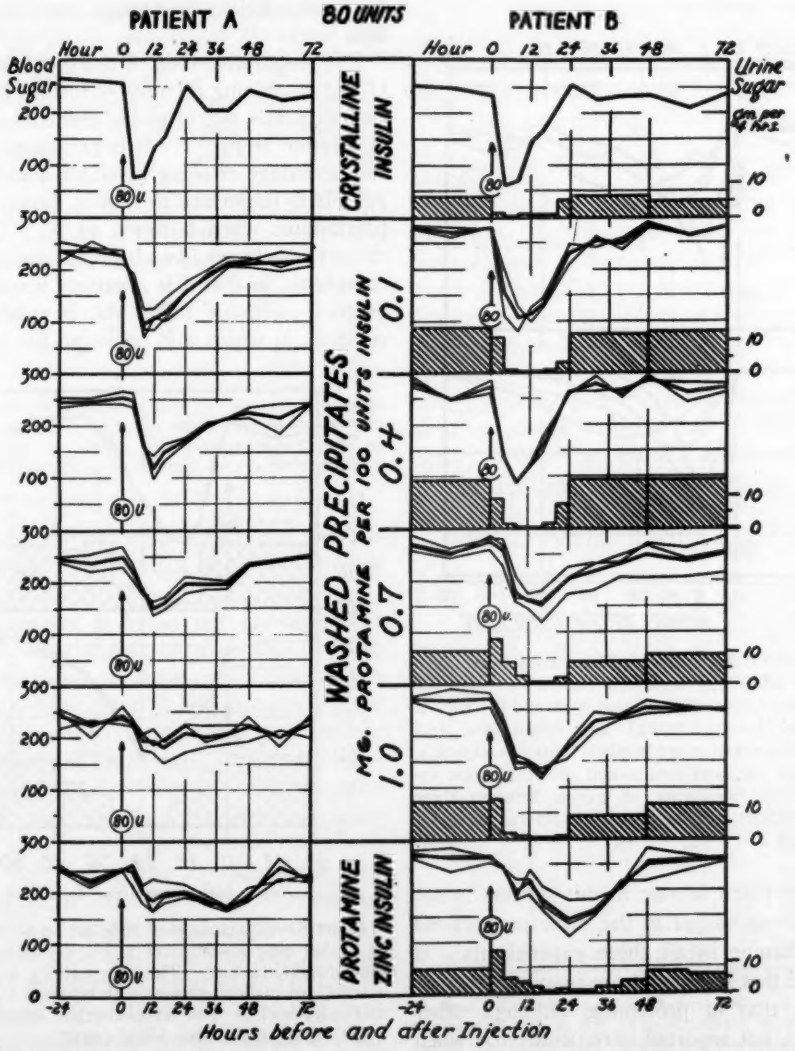

Chart 6.—Time action of saturated and unsaturated precipitates compared with that of standard insulin and protamine zinc insulin. The precipitates were made with 0.1, 0.4, 0.7 and 1.0 mg. of protamine per hundred units of insulin (1,000, 250, 143 and 100 units per milligram of protamine) at p_H 7.2. They were washed twice with p_H 7.2 buffer before resuspension and injection. The dosage of precipitate made with 0.1 mg. of protamine per hundred units (1,000 units per mg. of protamine) was quadrupled to obtain an estimated 80 unit effect. The observations were made on 2 diabetic patients with blood and urine sugar stabilized by four hour feedings as described in appendix IV. The cross-hatched blocks represent urine sugar; the curves, blood sugar.

fication saturated with insulin in firm combination. Insulin added in excess of this ratio remains soluble unless the acidity of the medium is increased, when it joins the precipitate in much higher ratios, as exhibited by the 4:1 simple mixture precipitating almost 500 units (20 mg.) of insulin per milligram of protamine at p_H 5.2.

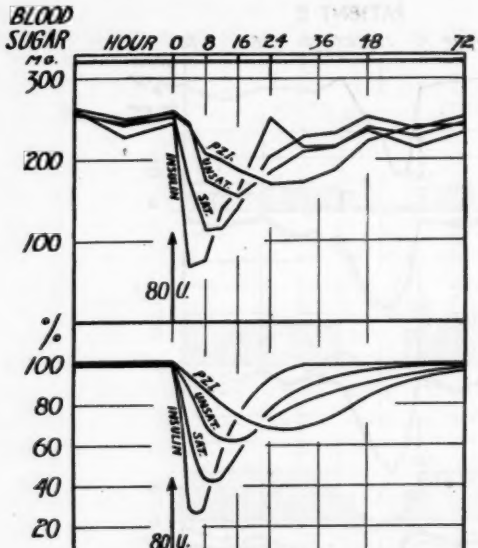

Chart 7.—Blood sugar curves from chart 6 averaged (above) and arbitrarily smoothed (below) to show intermediate time action of saturated (0.1 and 0.4 mg. of protamine per hundred units) and unsaturated (0.7 and 1.0 mg. of protamine per hundred units) precipitates, compared with standard insulin and protamine zinc insulin. Note the similarity of action between these washed precipitates and the simple unbuffered mixtures shown in chart 2.

The exact place of zinc in this scheme is not clear, as it was varied in the same proportions as the protamine in all these experiments. It is presumed that its importance is minor as compared with that of protamine, although other experiments, not reported here, show that when varied it may affect the physical character of the precipitates. Also, excesses of zinc have been observed to cause a relative increase in the soluble fractions of insulin in preparations such as those reported.

GLOBIN ZINC INSULIN

Along with the demonstrated usefulness of protamine zinc insulin modifications in the treatment of diabetes mellitus, interest has increased in the possibilities of another insulin modification with intermediate action, globin zinc insulin. Several authors have reported favorably on it.[16] None have made a careful comparison of it with modified protamine zinc insulins, and its position in therapy has not yet been established. It is of immediate practical importance to judge its action in comparison with that of the most efficient protamine zinc insulin available.

The preparation studied was globin zinc insulin U-80,[8] containing 80 units of insulin in combination with 3.04 mg. of globin and 0.24 mg. of zinc in solution at p_H 3.7. It is presumed to possess the advantage of being a soluble complex at this p_H. It is important to realize, however, that it precipitates when buffered to p_H 7.2. In this respect it behaves like all protamine zinc insulin complexes, in that it is relatively insoluble at the reaction of tissue fluids and is soluble in acid solution, in which it is packaged for injection.

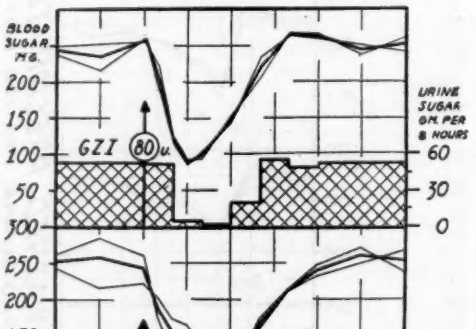

Chart 8.—Comparison of time action of 80 unit doses of globin zinc insulin and a 2:1 crystalline-protamine zinc insulin mixture. The observations were made on a stabilized diabetic patient with constant hyperglycemia and glycosuria. The cross-hatched blocks represent urine sugar; the curves, blood sugar.

Time-action curves resulting from injection of single 80 unit doses into a diabetic patient

16. Reiner, L.; Searle, D. S., and Lang, E. H.: Insulin Preparations with Prolonged Activity: I. Globin Insulin, Proc. Soc. Exper. Biol. & Med. **40**:171 (Feb.) 1939. Bauman, L.: Clinical Experience with Globin Insulin, Am. J. M. Sc. **198**:475-481 (Oct.) 1939. Duncan, G. G., and Barnes, C. A.: Action of Globin Insulin Compared with That of Crystalline, Unmodified, and Protamine Zinc Insulin, ibid. **202**:553-563 (Oct.) 1941. Bailey and Marble.[9b]

with stabilized blood and urine sugar levels are shown in chart 8. They are compared with the curves from similar doses of a 2:1 insulin–protamine zinc insulin mixture containing about 250 units per milligram of protamine.

The action of globin zinc insulin appears to be more prompt and intense and less prolonged than the 2:1 mixture which represents the approximate point of saturation of protamine with insulin at p_H 7.2.

This impression is borne out by two other comparisons of the two preparations. With single 60 unit doses, as shown in table 4, globin zinc insulin reduced the first day glycosuria more than the protamine zinc insulin modification. But the second and third day glycosuria, representing the sustained effects of the comparable single doses, was less with the protamine zinc insulin modification. Thus the total effect was about the same when the average three day excretion of sugar after insulin was compared with the average for four days without insulin. Sixty units of globin zinc insulin reduced the daily urinary excretion of sugar from 146 to 119 Gm. daily, and 60 units of the saturated protamine zinc insulin modification reduced it from 146 to 125 Gm. daily.

Both of these experiments were conducted on a patient whose glycosuria was stabilized by diet for testing with single doses. In another comparison (table 5), the effect of three daily doses of 40 units of each type of insulin was measured by the daily urinary excretion of sugar and overnight blood sugar levels of a patient with moderately severe diabetes on a fairly restricted diet in three meals daily. With globin zinc insulin the glycosuria was not fully controlled because the action of the insulin was not prolonged enough, as shown by the high fasting blood sugar level. On each of the last two days moderately severe hypoglycemic symptoms appeared in midafternoon. When the protamine zinc insulin modification was substituted, a transient rise in the excretion of sugar due to the lag in prompt effect was followed within three days by complete control of the glycosuria because the overnight level was controlled by the longer-acting modification. Hypoglycemic symptoms then occurred, and the dose was reduced to 32 units, which gave good control and no hypoglycemia.

These observations indicate that globin zinc insulin may be too prompt and brief in action to control severe diabetes satisfactorily in a single morning dose. The protamine zinc insulin modification compared with it may contain too little insulin for good control when diets high in carbohydrate are used. Yet Colwell and Izzo have used the latter preparation extensively in treatment of severe diabetes, with eminently satisfactory results [6f] provided the amounts of carbohydrate fed were kept at reasonable (equal to fat) levels. Furthermore, modification of a preparation such as this by adding insulin for increased promptness and intensity would be more expedient than slowing the action of globin zinc insulin.

Finally, the fact that globin zinc insulin, like clear, or acid, protamine zinc insulin, precipitates on injection into tissue fluids is a distinct disadvantage. The completeness of precipitation probably affects the promptness and hence the consistency of action of repeated doses of the

TABLE 4.—*Comparison of Effects of Single 60 Unit Doses of Globin Zinc Insulin and Unbuffered 2:1 Crystalline–Protamine Zinc Insulin on Glycosuria of Stabilized Diabetic Patient* *

	Globin Zinc Insulin			2:1 Mixture		
Experiment	1	2	Average	1	2	Average
	Grams of Sugar Excreted per 24 Hours					
Average for 4 days with no insulin			146			146
	Injection of Single 60 Unit Dose in Morning					
First day	57	68	63	95	101	98
Second day	153	82	117	159	128	143
Third day	167	185	176	142	126	134
Average for 3 days after insulin			119			125

* Girl aged 16, weighing 110 pounds (49.5 Kg.), with diabetes of three years' duration; diet, 229 Gm. carbohydrate, 73 Gm. of protein and 74 Gm. of fat, 1,874 calories, in six meals of approximately equal value daily, one every four hours day and night.

TABLE 5.—*Comparison of Globin Zinc Insulin and 2:1 Crystalline–Protamine Zinc Insulin Mixture in Routine Treatment of Diabetes Mellitus by Injection Once Daily (Weighed Diet Constant Throughout)* *

Day	Insulin: G. Z. I. Units	Insulin: 2:1 Units	24 Hour Urine Sugar, Gm.	Fasting Blood Sugar, Mg.	Insulin Shock, Time
1	0	0	46.5	202	..
2	0	0	32.7	182	..
3	0	0	46.4	208	..
4	40	0	10.5	202	0
5	40	0	20.2	193	3 p.m.
6	40	0	19.0	201	3 p.m.
7	0	40	33.6	172	0
8	0	40	6.1	100	0
9	0	40	0.9	85	3 p.m.
10	0	32	0.4	153	0

* Girl aged 16, weighing 110 pounds (49.5 Kg.), with diabetes of three years' duration, previously treated with protamine zinc insulin; diet, 63 Gm. of carbohydrate, 68 Gm. of protein and 68 Gm. of fat, 1,132 calories, in three meals daily.

same size and may lead to unpredictable variations in control, as reported by Bailey and Marble [9b] for clear protamine zinc insulin. Chart 9, showing time action curves for two identical doses of globin zinc insulin (and one dose of crystalline insulin for comparison) injected under the same conditions into a stabilized patient, suggests that this may be true. The variations in peak action and duration are greater than those seen with protamine zinc insulin modifications uniformly precipitated before injection. They might easily result in gross undulations in control on repeated daily injection for routine

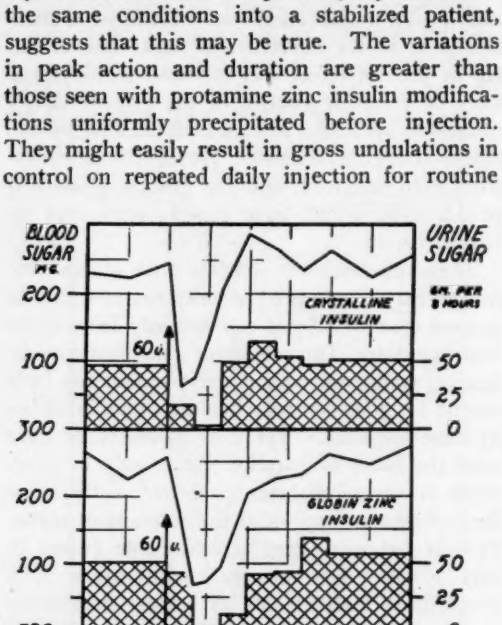

Chart 9.—Difference in response to two 60 unit doses of globin zinc insulin and to the same dose of crystalline insulin. The unequal action of globin zinc insulin could be due to difference in the rate of precipitation by tissue fluids in injection depots. The observations were made on a diabetic patient with constant blood and urine sugar stabilized by four hour meals of approximately equal value (table 4; appendix IV).

therapy. When viewed in this light, the presumed advantages of such a preparation in solution appear disadvantageous instead.

PRACTICAL CONSIDERATIONS

Certain constructive ideas emerge from the wealth of clinical experience and careful experimental studies of modified insulins.

The Ideal Insulin for Injection Once Daily.—Since any desired type of time activity may be obtained by varying the ingredients of known insulin combinations, it should be easy to select one of them as the ideal insulin for routine daily use in diabetes. It should release more insulin during the first twelve hours after injection than during the second, in order to correspond to day feeding and night fasting periods. Yet at twenty-four hours its action should be strong enough to avoid the necessity of a second daily dose in the evening. Also at its peak during the first twelve hours it should not be so intense in action as to cause hypoglycemia, particularly if the dextrose value of the diet is low, meals delayed or exercise excessive. It should be uniform in action from dose to dose, therefore preferably not precipitated by tissue fluids after injection. Other desirable but less essential qualifications should be relative freedom from allergic reactions and strength of 80 to 100 units per cubic centimeter because of relatively larger single dosage requirements.

Protamine Zinc Insulin: As now manufactured, this falls far short of these specifications. Used alone, it is suitable only for the milder forms of diabetes, which are easy to control with any type of depot insulin in a single dose daily. Too often it is necessary to supplement its action by separate injection or admixture with ordinary insulin, which leads to confusion, since added insulin is not reflected as such. Repeated doses are inconstant in effect, and allergic reactions are common. Use of this type of insulin as now prepared should be abandoned.

Globin Zinc Insulin: This has definite advantages over standard protamine zinc insulin. It gives accelerated, more intense action, which is desirable, and is relatively free from allergic effects. Yet it appears to be too prompt and intense in effect for ideal use, exhausting too much of its activity during the first twelve hours after injection and too often failing to cover the twenty-four hour period adequately. Its supposed advantage of homogeneity in solution could be duplicated with protamine zinc insulin or any of its modifications by acidification. This appears undesirable, since either acid solution would be precipitated by tissue fluids on injection, with resulting inconstancy of action from dose to dose, depending on completeness of precipitation in individual depots.

Saturated Protamine Zinc Insulin: Protamine zinc insulin modified by reduction of its protamine and zinc to about one third of its present content most nearly satisfies the specifications for an ideal insulin for once-daily use. In this form, containing about 0.4 mg. of protamine and 20 per cent as much zinc per hundred units, it

represents an insulin-saturated complex at p_H 7.2 the effect of which is intense enough during the first half-day after injection to cover moderate feeding values, weak enough during the second twelve hours to avoid hypoglycemia and yet strong enough to control the early morning sugar level in severe diabetes. In mild diabetes or with low carbohydrate diets hypoglycemia at the peak of the effect of the insulin is less likely than with globin zinc insulin. With higher carbohydrate diets it could be intensified by addition of more soluble insulin, which would be reflected directly in proportion to the insulin added because it is a saturated complex. For this reason it should be suspended in a slightly acid (p_H 5 to 6) buffer solution so that added insulin will join the insoluble complex, allowing it to retain its monophasic action. Such extemporaneous modification when desired could not be used to weaken and prolong the action of the more intense globin zinc insulin. Finally, because of its reduced protamine content, it is relatively free from allergic effects.

Such a modification can be prepared from insulins now available by mixing approximately two parts of solution of zinc insulin crystals with 1 part of protamine zinc insulin, both in the U-80 strength. This modification is stable when premixed in the ampoule, and its action does not vary appreciably when it is freshly mixed in the syringe on injection. All of its advantages have been proved by widespread clinical use.

It could replace protamine zinc insulin as now manufactured, because it controls mild diabetes as well and severe diabetes much better.[6f] Minor variations in protamine content would not affect its time action appreciably, since slight excesses or deficiencies in precipitated insulin do not change its action to a major degree (chart 6). Since prolonged activity due to slight excesses of protamine gains at the expense of promptness and intensity of action and vice versa, the overall action of such a preparation near the insulin saturation level is fairly uniform provided its total insulin content remains constant, even though the exact content of protamine varies somewhat from lot to lot. Variations in timing of protamine zinc insulin as now prepared are much greater, probably because of its insolubility and hence variable rate of release of insulin on depot injection.[17]

Adjustment of Diet.—In order to integrate the diet most efficiently with a preparation of this type it seems important to recognize two principles.

First, in order to utilize an insulin modification which is sufficiently active twenty-four hours after injection to control the hyperglycemia of severe diabetes, it is necessary to sacrifice some degree of intensity within the first twelve hours. Therefore diets extravagantly high in carbohydrate are not feasible. In ordinary dosage an insulin intense and prompt enough to control the glycosuria resulting from meals of high sugar value lacks satisfactory sustained action for severe diabetes. This appears to be the chief fault of globin zinc insulin.

Second, it is more convenient to vary the diet to meet individual differences in response of various diabetic patients to a fixed modification of insulin than to vary the insulin to meet a fixed diet. Experience with treatment of about 100 patients with widely differing grades of diabetes now shows this principle to be effective. It has been surprising to find that the differences are much less than anticipated. Ordinarily minor changes in the distribution of food are all that is necessary to accommodate different patients to the saturated protamine zinc insulin modification described herein. It should be emphasized, however, that diets extravagant in carbohydrates should not be used. Carbohydrate to fat ratios of about 1 to 1 in grams are most suitable. Insulin modifications possessing more intense action within the first twelve hours after their injection can carry more carbohydrate at mealtime, but good control of sugar twenty-four hours after their injection is impossible in severe diabetes, and hypoglycemic symptoms in the afternoon with exercise are more frequent and intense.

CONCLUSIONS

1. The activity of standard protamine zinc insulin is spread out too thin for most efficient once-daily use in cases of diabetes mellitus. On this account it permits glycosuria and hyperglycemia after meals, causes hypoglycemia during fasting, is too unpredictable in its overlapping effects and requires supplementation with ordinary insulin either by separate injection or by admixture. Because it is an insulin-unsaturated compound, mixtures made from it may be deceiving by not reflecting directly small amounts of insulin added to it.

2. The protamine and zinc now used in the preparation of protamine zinc insulin can combine two and one-half to three times as much insulin in buffered preparations and more when slightly acid. A saturated compound combines 250 to 300 instead of 100 units of insulin per milligram of protamine and 0.2 mg. of zinc at

17. Joslin, E. P.: Difficulties in the Use of Protamine Zinc Insulin, J. A. M. A. **110**:90-91 (Jan. 8) 1938.

p_H 7.2. It has a monophasic action in which it releases insulin at a more rapid rate than standard protamine zinc insulin. Therefore it is ideal for morning injection once daily because it controls glycosuria following meals better, is less likely to cause hypoglycemia during fasting, is more predictable in action and does not require supplementary insulin when used with diets of moderate carbohydrate value. When it is desirable to supplement it, added insulin is reflected directly in increased rapid effect. It is stable and practically free from allergic effects.

3. The action of globin zinc insulin is too quick and intense and not sustained enough for once-daily subcutaneous injection in cases of severe diabetes. Its supposed advantage of homogeneity by virtue of packaging in acid solution is a disadvantage instead, because its time action depends on the completeness of its precipitation on alkalinization after injection. Any protamine zinc insulin modification could be marketed in solution, but it is doubtful that that is desirable.

4. A single intermediate modification should be selected on the basis of greatest efficiency for the average diabetic patient. If prolonged enough in action it could replace standard protamine zinc insulin and not confuse the problem by addition of another insulin to the market. Modification of it would usually not be necessary with diets of moderate value. Adjustments in the distribution of food would accommodate to most variations in response of individual diabetic patients.

APPENDIXES

I. *Unbuffered Mixtures.*—Commercial supplies of solution of zinc insulin crystals U-80[14] and protamine zinc insulin U-80[15] were mixed in the proportions indicated. The soluble and insoluble portions were separated by thorough centrifuging and decanting, except in the case of mixtures containing more than 5 parts of insulin to 1 part of protamine zinc insulin. With these, colloidal precipitation prevented separation of the fractions and interfered with accurate estimations of insulin in the supernatants. These preparations also involved obvious solution of the protamine zinc insulin precipitates into the supernatant fraction because of their relatively high acidity.

II. *Volume of Precipitate.*—The volumes of precipitate were obtained by thorough centrifuging of each preparation in ordinary hematocrit tubes. This tube is 10 cm. long and holds 707 cu. mm. by volume. It is graduated into one hundred divisions, each of which represents 7.07 cu. mm. of volume. The levels of precipitate were read to the closest 0.1 cu. mm. division, which was corrected by calculation to give the volume of precipitate for each cubic centimeter of the preparation studied.

III. *Insulin in Supernatant.*—Insulin in solution was estimated by an adaptation of the Mirsky and Anson method[18] for determination of cystine colorimetrically. Five cubic centimeters of the unknown supernatant was mixed in a test tube with 2 cc. of freshly prepared 10 per cent solution of sodium cyanide, used for reduction of cystine by Sullivan and Hess.[19]

After the mixture had stood for twenty minutes at room temperature, 4 cc. of 6 times molar urea solution was added with mixing to prevent precipitation. Then 3 cc. of the phosphotungstic acid reagent used in the determination of uric acid by Folin and Denis[20] was added. The maximum clear blue color developed when the tubes stood for ten minutes in a hot water bath. It was read colorimetrically against a suitably diluted standard of solution of zinc insulin crystals[14] treated in the same manner. Insulin in units per cubic centimeter was calculated from the colorimetric and dilution factors in the usual manner.

This method has been found accurate within a 10 per cent limit of error by repeated testing of known preparations of insulin in various dilutions and at different p_H values. The supernatants studied varied from the insulin standards in their buffer and zinc content and possibly also contained faint traces of protamine. These variables were eliminated as possible sources of error by testing known insulin preparations containing them and by testing blanks containing them without insulin. None of them varied the color obtained from known insulin preparations, and none produced any great error in blank determinations without insulin.

There was good agreement between the results obtained by this method and those obtained by the method described in appendix V. The amounts of insulin estimated by both methods agreed with the blood sugar-reducing action of unknown supernatants, as described in appendix IV. These methods may be considered accurate within 10 per cent when precipitation is avoided and when the unknowns contain no other possible source of cystine than insulin.

IV. *Curves of Values for Blood and Urine Sugar.*—The methods used in obtaining these curves for stabilized diabetic patients have been described in detail by Colwell, Izzo and Stryker[5] and Colwell and Izzo[6] in previous reports.

In brief, patients with moderate grades of diabetes were forced to have high blood and urine sugar values at constant levels by omission of insulin and ingestion of approximately equal meals of high carbohydrate value every four hours night and day. Samples of blood taken every four to twelve hours before meals and of urine obtained in four to twelve hour collections were analyzed for sugar by conventional methods. When reasonable constancy was observed, single doses of the unknown insulins tested were injected subcutaneously and the blood and urine sugar determined at frequent intervals as before until the effects waned and the previous control levels were reestablished.

In this way overlapping effects are avoided and a true picture of the time action and potency of the insulins tested is obtained. The curves illustrate the pharmacology of the various insulins studied, from which therapeutic applications may easily be deduced.

18. Mirsky, A. E., and Anson, M. L.: Sulfhydryl and Disulfide Groups of Proteins: I. Methods of Estimation, J. Gen. Physiol. **18**:307-323 (Jan.) 1935.

19. Sullivan, M. X., and Hess, W. C.: Studies on Biochemistry of Sulphur: VIII. The Cystine of Purified Proteins, Pub. Health Rep., 1930, supp. 86.

20. Folin, O., and Denis, W.: On Phosphotungstic-Phosphomolybdic Compounds as Color Reagents, J. Biol. Chem. **12**:239-243, 1912.

V. *Semi-Quantitative Method for Estimation of Soluble Insulin.*—One cubic centimeter of the unknown solution containing insulin was brought to a boil with 0.1 cc. of 10 per cent sodium hydroxide. One drop of saturated aqueous solution of lead acetate was added. In the presence of sulfur a brownish black precipitate of lead sulfide appears,[21] the amount of which can be judged roughly by comparison with a suitably diluted standard containing a known amount of insulin treated in the same manner.

In the presence of any other source of sulfur than insulin this method is worthless for estimating insulin. In the presence of protamine white flocculent precipitates occur which obscure the lead sulfide. The sulfide precipitate interferes with quantitative colorimetric comparisons with known insulin standards, but naked eye comparisons are accurate within about 5 units per cubic centimeter of unknown tested. Amounts of insulin as small as 1 unit per cubic centimeter give no precipitate.

Results by this method agree with those obtained by blood sugar curves, as described in appendix IV and illustrated in chart 3. In that chart the results in units per cubic centimeter first obtained by this method are superimposed on the blood sugar curves obtained after injection of 1 to 2 cc. of the same material. The results are also in agreement with the colorimetric method described in appendix III, but that method is quantitative and sensitive to greater dilutions (1 to 2 units per cubic centimeter) of insulin.

21. Gortner, R. A.: Outlines of Biochemistry, New York, John Wiley & Sons, Inc., 1929, p. 325.

EFFECT OF SULFONAMIDE COMPOUNDS ON TRANSIENT BACTEREMIA FOLLOWING EXTRACTION OF TEETH

I. SULFANILAMIDE

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PHILADELPHIA

Bacteremia has long been known to follow almost all surgical procedures, from curettage of the uterus [1] and operations on the urethra [2] to abdominal operations such as simple appendectomy.[3] In 1932 Richards [4] reported the occurrence of bacteremia following irritation of foci of infection, such as joints, tonsils, prostates, furuncles and gums. Bacteremia following extraction of teeth has been reported by numerous investigators.[5] It remained for Okell and Elliott [5a] to demonstrate that bacteremia follows extraction of teeth more frequently than had hitherto been realized. Of cultures of blood taken immediately after dental extraction in 138 cases, 60.9 per cent revealed bacteria. In 1939 Elliott,[5f] using an improved technic and selected patients, was able to obtain positive results from 86 per cent of cultures in 21 cases.

One of the more unusual features in the work of Okell and Elliott [5a] was that of 110 persons undergoing extraction 10.9 per cent were found to have bacteria in the control cultures (those made immediately before the teeth were extracted). At that time no adequate explanation could be advanced for this phenomenon. Later experiments,[6] however, make it apparent that minor degrees of trauma, such as chewing, are sufficient to initiate repeated showers of organisms into the blood stream. This occurs especially in the presence of periodontal disease and possibly even in its absence.

Whether such dental bacteremia can ever be prevented by the prophylactic use of sulfonamide compounds has not been sufficiently demonstrated.[7]

This study endeavors to show the effect of sulfanilamide on bacteremia following the extraction of teeth.

PROCEDURES

Ten cubic centimeters of blood was drawn from the median basilic vein in the antecubital space of either arm, through a 22 gage needle, into a sterile 20 cc. syringe. Eight cubic centimeters of this was divided equally between two 100 cc. Erlenmeyer flasks, each containing 20 cc. of medium (brain-heart infusion broth [Difco] fortified with 0.033 per cent sodium polyanethol-

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‡ This paper is abstracted from a thesis submitted to the faculty of the Graduate School of Medicine of the University of Pennsylvania toward the requirements for the degree of Master of Medical Science (M.Sc.Med.) for graduate work in Internal Medicine.

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3. Seifert, E.: Bacteremia After Operations, Arch. f. klin. Chir. **138**:565, 1925.

4. Richards, J. H.: Bacteremia Following Irritation of Foci of Infection, J. A. M. A. **99**:1496 (Oct. 29) 1932.

5. (*a*) Okell, C. C., and Elliott, S. D.: Bacteremia and Oral Sepsis, Lancet **2**:869, 1935. (*b*) Fish, E. W., and MacLean, I.: Distribution of Oral Streptococci in the Tissues, Brit. Dent. J. **61**:336, 1936. (*c*) Burket, L. W., and Burn, C. G.: Bacteremias Following Dental Extraction: Demonstration of Source of Bacteria by Means of a Non-Pathogen (Serratia Marcesens), J. Dent. Research **16**:521, 1937. (*d*) Marseille, A.: Bacteremia After Extraction of Teeth, Geneesk. tijdschr. v. Nederl.-Indië **77**:2491, 1937. (*e*) Southworth, H., and Flake, C. G.: Blood Cultures After Tonsillectomy, Am. J. M. Sc. **195**:667, 1938. (*f*) Elliott, S. D.: Bacteremia and Oral Sepsis, Proc. Roy. Soc. Med. **32**: 747, 1939. (*g*) Palmer, H. D., and Kempf, M.: Streptococcus Viridans Bacteremia Following Extraction of Teeth: Case of Multiple Mycotic Aneurysms in the Pulmonary Arteries, J. A. M. A. **113**:1788 (Nov. 11) 1939. (*h*) Bartels, H. A.: Review of Recent Literature Dealing with Transient Bacteremias, Am. J. Orthodontics **26**:366, 1940. (*i*) Murray, M., and Moosnick, F.: Incidence of Bacteremia in Patients with Dental Disease, J. Lab. & Clin. Med. **26**:801, 1941. (*j*) Paquin, O. A., Jr.: Bacteremia Following Removal of Diseased Teeth, J. Am. Dent. A. **28**:879, 1941. (*k*) Northrop, P. M., and Crowley, M. C.: The Prophylactic Use of Sulfathiazole in Transient Bacteremia Following the Extraction of Teeth: A Preliminary Report, J. Oral Surg. **1**:19, 1943; (*l*) A Preliminary Report on the Prophylactic Use of Sulfathiazole on Transient Bacteremia Following Extraction of Teeth, New York J. Dent. **13**:97, 1943.

6. Round, H.; Kirkpatrick, H. J. R., and Hails, C. G.: Further Investigations on Bacterial Infections of the Mouth, Proc. Roy. Soc. Med. **29**:1552, 1936. Murray and Moosnick.[5i]

7. Taran, L. M.: Dental Care of Children with Heart Disease, Distr. Dent. Soc. State New York **28**: 93, 1942. Northrop and Crowley.[5k]

sulfonate[8]).[9] The other 2 cc. was placed in a 25 cc. test tube containing 10 cc. of medium (for anaerobic culture). From patients receiving sulfanilamide an additional 5 cc. was taken. This sample was citrated, and the level of sulfanilamide was determined.[10] The blood was withdrawn at the following intervals: immediately before extraction, immediately after extraction and ten minutes after extraction. The cultures were incubated at 37 C. and examined at twenty-four hour intervals for seven days. Only cultures that became cloudy, showed change in color or an odor or otherwise appeared to possess a growth were opened. All culture flasks were opened and smears made of the contents at the end of seven days. All suspicious specimens were subcultured before being discarded.

Anaerobic technics were followed according to the Brewer-Brown method.[11]

MATERIAL

Clinical material was taken from the dental outpatient clinic and wards of the Jewish Hospital, Philadelphia. A total of 63 patients were examined. Patients were taken at random, the only requirement being that two or more teeth were to be extracted. The 63 patients were divided into two groups that were studied concurrently. Three were rejected. This left a group of 30 control patients and a group of 30 patients that received sulfanilamide. The first 10 patients were all controls, but thereafter selections were made so that the two groups were approximately equal. The extractions were done in the morning, usually not more than 3 patients being treated at a time.

The ages ranged from 19 to 70, the bulk of patients being above 40 years. There were 25 women and 35 men.

The gums were graded on a basis of the severity of the gingival disease: periodontoclasia, class I, II or III.[12] In class I there was no apparent disease of the gums; in class II, mild to moderate disease, and in class III, severe disease.

All patients were the subject of a brief history taking and physical examination. The blood pressure was recorded and the heart and lungs examined. The patients receiving sulfanilamide had blood counts taken after the completion of the administration of the drug and the extraction of teeth. These counts are not listed, as they revealed nothing significant. Extractions ranged from two to ten teeth. An average of approximately four teeth were removed from the subjects in the control group (I), and approximately four and eight-tenths teeth were extracted for the group given sulfanilamide (II). The usual method of extraction was employed, with the elevator and forceps technic.

All the patients were anesthetized with approximately 2.5 cc. of a 2 per cent solution of procaine hydrochloride without epinephrine. Anesthesia was induced either by conduction or by infiltration, depending on the location of the teeth. Procaine without epinephrine was used so that it would not interfere with the dispersal of organisms from the gingival trough.[5e]

All patients were given the same dosage, a total of 110 grains (7.35 Gm.) in the twenty-four hours preceding extraction. This was given as follows: 20 grains (1.35 Gm.) four times daily the day before the extraction and 30 grains (2 Gm.) four hours prior to the extraction.

Despite the fact that all patients received the same dosage, the level of sulfanilamide in the blood varied from 5.0 to 11.0 mg. per hundred cubic centimeters (table 3). The average level for the 30 patients receiving sulfanilamide was 7.5 mg. per hundred cubic centimeters. Three patients with a level below 5 mg. were discarded because it was felt that a maximum effect could be obtained only with a level above 5 mg. Spink[13] has recommended a slightly higher figure, 7 mg. per hundred cubic centimeters. Although it is feasible to obtain the level recommended by Spink as an average, it was found that ambulatory patients could not tolerate any higher doses. The rejected patients had not taken the full dose prescribed for them; hence the insufficient blood level.

No severe reactions were noted. The majority of our patients complained of mild vertigo and nausea. Nearly all displayed some degree of cyanosis.

RESULTS

Of the cultures taken prior to extraction for the control group, one was positive. Staphylococcus albus was the organism that was recovered. For the group given sulfanilamide all cultures taken before the extraction were negative.

Of the cultures taken from the control subjects immediately after the extraction, 83.3 per cent or 25 of 30, were positive. The organisms isolated were viridans streptococci (21 times), Staph. albus (4 times), pneumococci (nontypable) (3 times) and nonhemolytic streptococci (once). Of the cultures taken from the sulfanilamide-treated group immediately after the extrac-

8. At first duplicate cultures were made of all aerobic specimens, but later in the course of study (because of the war) difficulty was experienced in obtaining the sodium polyanetholsulfonate from Switzerland, where it was manufactured. As a result, only one sample was taken for the preextraction and the ten minute postextraction culture, instead of their being run in duplicate. The sodium polyanetholsulfonate was supplied by Hoffman-La Roche, Inc.

9. (*a*) Massa, M., and Battistini, G.: Anticomplementary Action and Inhibition of Bactericidal Power of Blood by Sodium Polyanetholsulfonate Used in Hemoculture, Zentralbl. f. Bakt. (Abt. 1) **131**:241, 1934. (*b*) Elliott, S. D.: Bacteremia Following Tonsillectomy, Lancet **2**:589, 1939. (*c*) Baiocchi, P.: Contributo allo studio delle batteriemie chirurgiche con emoculture comparate; batteriemia pre- e post-operatoria negli interventi asettici, Gior. di batteriol. e immunol. **21**:63, 1938. (*d*) von Haebler, T., and Miles, A. A.: The Action of Sodium Polyanetholsulphonate ("Liquoid") on Blood Cultures, J. Path. & Bact. **46**: 245, 1938. (*e*) Hoare, E. D.: The Suitability of "Liquoid" for Use in Blood Culture Media, with Particular Reference to Anaerobic Streptococci, ibid. **48**: 573, 1939.

10. Bratton, A. C., and Marshall, E. K., Jr.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**:537, 1939.

11. Brewer, J. H., and Brown, J. H.: A Method for Utilizing Illuminating Gas in the Brown, Fildes and McIntosh or Other Anaerobe Jars of the Laidlaw Principle, J. Lab. & Clin. Med. **23**:870, 1938.

12. Eighth International Dental Congress, Paris, 1931, sect. 4.

13. Spink, W. W.: Sulfanilamide and Related Compounds in General Practice, Chicago, The Year Book Publishers, Inc., 1941.

tion, 76.7 per cent, or 23 of 30, were positive. Recovered were viridans streptococci (20 times), pneumococci (nontypable) (twice), Staph. albus haemolyticus (once) and nonhemolytic streptococci (once).

In the cultures for the control, of the 30 taken ten minutes after the extraction, 33.3 per cent, or 10 of 30, were positive. The organisms isolated were viridans streptococci (9 times), and Staph. albus (twice). Of the cultures from the sulfanilamide-treated group, 13.3 per cent, or 4 of 30, were positive. The organisms recovered were viridans streptococci (twice), Staph. albus haemolyticus (once) and pneumococci (nontypable) (once).

Of the anaerobic cultures taken from the control group immediately after the extraction, 62.9 per cent, or 17 of 27, exhibited growth. Viridans streptococci were recovered 15 times, Staph. albus twice and nonhemolytic streptococci twice. Of the anaerobic cultures from the sulfanilamide-treated group 40 per cent, or 12 of 30, were positive. Viridans streptococci were recovered 11 times and hemolytic streptococci once.

TABLE 1.—*Incidence of Bacteremia*

No. of Patients	Group	Before Extraction	Immediately After Extraction	Ten Minutes After Extraction
	Aerobic Cultures			
30	Control (I)	1 (3.3%)	25 (83.3%)	10 (33.3%)
30	Sulfanilamide-treated (II)	0 (0.0%)	23 (76.6%)	4 (13.3%)
	Anaerobic Cultures			
27	Control (I)	0 (0.0%)	17 (62.9%)	3 (11.1%)
30	Sulfanilamide-treated (II)	0 (0.0%)	12 (40.0%)	1 (3.3%)

Of the anaerobic cultures taken from the control group ten minutes after the extraction, 11.1 per cent, or 3 of 27, were positive. Viridans streptococci were isolated twice and Staph. albus in the other instance.

In the ten minute anaerobic cultures for the sulfanilamide-treated group there was only 3.3 per cent, or 1, positive culture. Viridans streptococci were isolated (tables 1, 2 and 3).

Although the administration of sulfanilamide produced no significant difference in the number of positive cultures immediately after the extraction of two or more teeth, ten minutes later a significant effect was observed. Whereas 33.3 per cent of the cultures for the control group were positive, only 13.3 per cent of those for the sulfanilamide-treated group were positive. Thus the administration of sulfanilamide was able to effect a 20 per cent decrease in the ability of organisms to grow after circulating in a blood stream with 5 mg. or more of sulfanilamide per hundred cubic centimeters of blood.

Generally speaking, the anaerobic cultures were disappointing. Only 62.9 per cent, or 17, of 27 cultures taken immediately after extraction were positive, in comparison with 83.3 per cent in the aerobic group, a decrease of 20.6 per cent. When sulfanilamide was administered, 76.6 per cent of the aerobic cultures were positive. Under anaerobic conditions, only 40.0 per cent still remained positive, a decrease of 36.6 per cent.

Although the green-producing streptococci are facultative anaerobes, they are supposed to grow well under strict anaerobic conditions, but it should be remembered that they are a heterogeneous group and culture characteristics may vary widely.[14] Perhaps, as suggested by Hoare,[9e] sodium polyanetholsulfonate (liquoid) inhibits growth of anaerobic streptococci; thus sodium citrate broth might result in a greater percentage of positive cultures. Spink [13] pointed out the fact that in vitro sulfanilamide is bacteriostatic to a marked degree in an anaerobic medium. Another consideration is that only 2 cc. of blood was cultured anaerobically, while 8 cc. was cultured aerobically. Thus, under conditions in which organisms are so few, definitely being less than 1 bacterium per cubic centimeter, inevitably there must be a lesser number of positive cultures.

On no occasion in our studies was a growth obtained under anaerobic conditions when the organism failed to grow aerobically, although twice viridans streptococci grew in the spray [14e] dish when pneumococci [14d] and staphylococci had grown in the aerobic medium (patients 11 and 26 in table 2).

Classically streptococci are divided into alpha, beta and gamma groups.[14e] Alpha streptococci are the organisms that produce greening (hence viridans streptococci) and compose in the main a heterogeneous group. Occasional fermentation reactions were done, but they were rarely completely satisfactory. In all cases, in order to differentiate streptococci from pneumococci, inulase

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TABLE 2.—*Data on Control Group (I)* *

No.	Patient: No.	Initials	Age	Sex	Peri-odonto-clasia	No. of Teeth	Aerobic: 1	2A	2B	3A	3B	Anaerobic: 1	2	3
1	1	W. M.	65	M	III	6 (3 roots)	—	v.S	nh.S	v.S	v.S	0	0	0
2	2	E. C.	56	M	III	6	—	v.S	—	v.S	—	0	0	0
3	3	M. G.	58	M	III	3	—	—	—	St.al.	—	0	0	0
4	4	C. G.	63	M	I	2 (periapical infection)	—	v.S	v.S	v.S	—	—	v.S, St.al.	—
5	5	A. S.	45	M	III	5	—	v.S	—	—	—	—	v.S	—
6	6	S. S.	63	M	III	5	—	v.S	v.S	—	—	—	nh.S	—
7	7	J. M.	54	M	III	5	—	v.S, St.al.	v.S	St.al.	v.S	—	v.S	St.al.
8	8	J. O.	21	F	I	3	—	v.S	v.S	St.al.	—	—	v.S	—
9	9	D. G.	26	M	II	4	—	v.S	v.S	—	—	—	v.S	—
10	10	B. R.	51	M	I	3 roots	—	v.S	—	—	—	—	nh.S	—
11	11	M. P.	55	M	III	6	—	Pn.	St.al.	—	—	—	v.S	—
12	14	E. F.	42	M	I	2 roots	—	—	—	—	—	—	—	—
13	15	H. P.	47	M	II	4	—	v.S	v.S	v.S	0	—	v.S	—
14	16	D. G	47	M	II	4	—	v.S, St.al.	v.S, St.al.	v.S	0	—	v.S	v.S
15	26	I. K.	50	M	I	2 roots (periapical infection)	St.al.	St.al.h.	—	—	—	—	v.S	—
16	27	E. F.	67	M	III	4	—	—	—	—	—	—	—	—
17	29	A. F.	48	F	I	4	—	v.S	v.S	v.S	v.S	—	v.S	v.S
18	35	F. S.	55	F	I	4	—	—	—	—	—	—	—	—
19	36	B. C.	43	M	II	2 roots	—	v.S	v.S	—	—	—	v.S	—
20	39	P. S.	55	F	I	5	—	v.S	v.S	v.S	v.S	—	v.S	—
21	43	J. A.	78	F	III	4	—	v.S	v.S	—	—	—	v.S	—
22	44	M. S.	58	M	III	7	—	—	—	—	—	—	—	—
23	45	J. M.	40	F	II	5 (2 roots)	—	Pn.	Pn.	—	—	—	—	—
24	46	W. R.	49	M	II	4	—	v.S	v.S	—	0	—	—	—
25	47	O. G.	51	M	II	3	—	v.S	v.S	—	0	—	—	—
26	49	M. S.	41	F	III	2	—	v.S	v.S	—	0	—	—	—
27	50	J. L.	65	M	II	6	—	v.S	v.S	—	0	—	v.S	—
28	52	G. N.	36	F	II	2	—	v.S	v.S (1½ min. late)	—	0	—	v.S	—
29	54	M. D.	43	M	II	3	—	Pn.	—	—	0	—	—	—
30	57	B. M.	37	F	I	3 roots	—	v.S	v.S	v.S	0	—	—	—

* The symbols and abbreviations have the following significance: —, negative; 0, no culture obtained; v.S, viridans streptococci; Pn., pneumococci; nh.S, nonhemolytic streptococci; St.al., Staphylococcus albus; St.al.h., Staph. albus haemolyticus.

TABLE 3.—*Data on Sulfanilamide-Treated Group (II)* *

No.	Patient: No.	Initials	Age	Sex	Peri-odonto-clasia	No. of Teeth	Blood sulf.	Aerobic: 1	2A	2B	3A	3B	Anaerobic: 1	2	3
1	12	A. S.	45	M	III	4	6.1	—	v.S	v.S	—	—	—	v.S	—
2	13	W. M.	65	M	III	6 (1 root)	8.0	—	v.S	—	—	—	—	v.S	—
3	17	F. D.	24	F	I	4	6.8	—	v.S	—	—	0	—	S.h	—
4	18	E. F.	67	M	III	4	8.8	—	St.al.hem. Strept.†	—	—	0	—	v.S	—
5	19	T. S.	52	F	II	3	6.4	—	v.S	v.S	—	0	—	v.S	—
6	20	B. G.	43	F	II	2	8.8	—	v.S	v.S	—	0	—	v.S	—
7	21	R. S.	45	F	I	2 roots	10.8	—	v.S	v.S	—	—	—	v.S	—
8	23	N. W.	46	F	III	4	6.4	—	—	—	—	—	—	—	—
9	24	C. L.	56	M	III	4	5.6	—	v.S	v.S	—	—	—	v.S	—
10	28	L. M.	49	F	III	3	9.6	—	—	—	—	—	—	—	—
11	30	K. D.	52	F	III	2	6.0	—	v.S	—	—	—	—	v.S	—
12	31	E. B.	19	F	I	3	8.8	—	v.S	—	v.S	—	—	—	—
13	32	N. B.	52	M	II	5	6.4	—	v.S	—	St.al.hem.	—	—	—	—
14	33	M. R.	45	F	II	6	10.4	—	v.S	v.S	—	—	—	—	—
15	34	T. S.	48	F	II	3	9.6	—	—	—	—	—	—	—	—
16	37	W. L.	58	M	III	4	6.4	—	v.S	v.S	—	—	—	—	—
17	38	L. K.	36	M	I	4	5.6	—	v.S	v.S	—	—	—	v.S	—
18	40	E. N.	48	F	III	3	6.4	—	v.S	v.S	v.S	v.S	—	v.S	v.S
19	41	J. K.	60	M	I	7	6.4	—	—	—	—	—	—	—	—
20	42	F. G.	33	M	II	10	5.8	—	—	—	—	—	—	—	—
21	48	M. O.	37	F	II	6	8.0	—	v.S	v.S	—	0	—	—	—
22	51	A. R.	55	M	III	6	5.6	—	Pn.	Pn.	—	0	—	—	—
23	53	G. M.	69	M	III	6	6.5	—	v.S	v.S	—	0	—	v.S	—
24	54	M. F.	70	F	III	3	11.0	—	Pn.	Pn.	Pn.	0	—	—	—
25	56	M. D.	36	F	III	6	8.0	—	—	—	—	0	—	—	—
26	58	J. V.	67	M	II	6	5.0	—	v.S	—	—	0	—	—	—
27	60	B. B.	57	F	III	7 (2 roots)	7.2	—	—	—	—	0	—	—	—
28	61	A. Z.	51	M	I	6 (3 with periapical inf.)	5.0	—	v.S	v.S	—	0	—	—	—
29	62	M. B.	56	F	III	6 (2 roots)	10.0	—	v.S	v.S	—	0	—	—	—
30	63	J. T.	52	M	II	6	7.1	—	v.S	v.S	—	0	—	—	—

* The symbols and abbreviations have the following significance: —, negative; 0, no culture obtained; v.S, viridans streptococci; Pn., pneumococci; St.al.hem., Staph. albus haemolyticus; S.h., hemolytic streptococci.
† Unable to isolate strain of streptococcus.

activity and bile solubility tests were performed. Solovey [14b] called attention to the fact that serologic methods have shown the viridans streptococci to be a heterogeneous group of organisms. She pointed out that there is no serologic difference between strains of viridans streptococci isolated from cultures of subacute bacterial endocarditis, human throats and extracted teeth.

In our series of experiments viridans streptococci were recovered 81 times (table 4).

The other types of streptococci were rarely seen, the alpha, or nonhemolytic, variety being recovered four times and the beta, or hemolytic, variety being seen only once. The two other organisms found with some degree of frequency were pneumococci and Staph. albus. In all, pneumococci were recovered from the blood stream 7 times. They tended to be atypical, with aberrations of the capsule, and in every case resisted typing with type-specific serums. This indicates that they were avirulent, saprophytic inhabitants of the oral cavity.

TABLE 4.—*Organisms Recovered*

	Before Extraction		Immediately After Extraction		Ten Minutes After Extraction			
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Total	
Control Group (I)								
Viridans streptococci	0	0	21	15	9	2	47	
Staphylococcus albus	1	0	4	1	2	1	9	
Pneumococci	0	0	3	1	0	0	4	
Nonhemolytic streptococci	0	0	1	2	0	0	3	
Total	1	0	29	19	11	3	63	
Sulfanilamide-Treated Group (II)								Grand Total
Viridans streptococci	0	0	20	11	2	1	34	(81)
Staphylococcus albus	0	0	1	0	1	0	2	(11)
Pneumococci	0	0	2	0	1	0	3	(7)
Hemolytic streptococci	0	0	0	1	0	0	1	(1)
Nonhemolytic streptococci	0	0	1	0	0	0	1	(4)
Total	0	0	24	12	4	1	41	

The only type of Staphylococcus found was the albus variety, and it frequently displayed hemolysis. In all it was recovered 11 times, mainly from the control group. Although we did not use the coagulase test [15] uniformly, we did find that of the organisms tested all were avirulent. For our control series we found only one positive growth in the preoperative culture. Although they are considered by some to be a contaminant,[5f,1] Burn and Burket [16] in postmortem studies of the periapical tissues and the blood stream showed staphylococci to be present in 48 per cent of their positive cultures, while the same organisms were obtained in 42 per cent of cultures taken from the blood stream at the same time. Thus, the staphylococci cannot be considered contaminants but should be considered as inhabitants of the oral cavity that gain access to the circulatory system.

Recently it has been demonstrated that paraaminobenzoic acid exerts an inhibitory effect on sulfanilamide.[17] It has been suggested that it should be added to cultures when sulfanilamide is present in order to counteract bacteriostasis and allow the organisms present to grow. This was decided against, since it was felt that it would interfere with the correlation and that in vivo conditions would not be duplicated. Even if organisms gain access to the circulation, the important fact to know is whether they are capable of growing under the conditions present at that time. The interesting finding was that there was no significant difference in the results between the cultures containing sulfanilamide (76.6 per cent) and the controls (83.3 per cent). Moreover, it was determined that the sulfanilamide was stable, being still present in the same concentration in the flask at the end of seven days.

Moreover, occasional control subjects had samples of blood taken to determine if any procaine had entered the blood stream, since its action is similar to that of paraaminobenzoic acid.[18] In all instances the findings were completely negative.

COMMENT

The problem of isolating organisms circulating in the blood stream is one of peculiar difficulty. Culture technics must be of unusual sensitivity, since it is apparent that the offending bacteria are few. At first, 1 cc. of citrated blood was plated with 10 cc. of agar. Such cultures were

15. Fairbrother, R. W.: Coagulase Production as a Criterion for the Classification of Staphylococci, J. Path. & Bact. **50**:83, 1940.

16. Burn, C. G., and Burket, L. W.: Comparative Bacteriologic Studies of Human Blood, Viscera and Teeth Obtained at Necropsies, Arch. Path. **25**:643 (May) 1938.

17. Woods, D. D.: The Relation of Para-Aminobenzoic Acid to the Mechanism of the Action of Sulfanilamide, Brit. J. Exper. Path. **21**:74, 1940.

18. de Waal, H. L.; Kanaar, A. C., and McNaughtan, J.: Antisulphanilamide Action of Procaine in Vivo, Lancet **2**:724, 1942.

negative, although broth cultures were positive, which indicates that the organisms were fewer than 1 per cubic centimeter of blood. A further substantiation is the finding in duplicate cultures of 1 positive and 1 negative culture (table 2). If there is only 1 organism per 8 cc., then 1 flask must show no growth, as was repeatedly observed. Thus, unless bacteria are given every chance to grow by the most sensitive methods of blood culture, negative results will be obtained. We believe that the low percentage of positive cultures obtained by many workers is due to the failure to use a sufficiently sensitive medium and not to a difference between general and local anesthesia.[5c]

In 1934, Massa and Battistini,[9a] employing sodium polyanetholsulfonate in 0.1 to 0.2 per cent concentration, found this method superior to accepted blood culture technics then in use. Baiocchi [9c] found 50 per cent positive cultures in cases of postoperative bacteremia with the use of sodium polyanetholsulfonate ("liquoid") whereas only 15 per cent were positive when dextrose broth was employed. Von Haebler and Miles [9d] using sodium polyanetholsulfonate in a concentration of 0.03 to 0.05 per cent, found it to be an effective anticoagulant. They also found it to impair or destroy the bactericidal power of normal human blood for many pathogenic bacteria, including viridans streptococci. The inhibition of this bactericidal power was believed due to neutralization both of complement and of beta lysin. Hoare [9e] arrived at the same conclusion but noted that sodium polyanetholsulfonate was unfavorable to the growth of anaerobic streptococci of five strains examined.

Considering the myriad organisms that inhabit the mouth and the ease with which they can enter the circulation, the surprising finding is that cultures are not uniformly positive after extractions. This may be ascribed in part not only to the efficient clearing mechanism that exists within the body but to the difficulties of culturing.

The speed of the clearing mechanism is shown in the control group, 83.3 per cent of whose cultures were positive immediately after the extraction. In ten minutes only 33.3 per cent were still positive. Although we did not take cultures after the ten minute interval, it has been shown by numerous investigators [5] that beyond ten minutes and up to thirty minutes only occasional cultures remain positive.

Reichel [19] demonstrated that there is apparently no limit to the number of bacteria that can be removed and that all types of bacteria can be removed simultaneously. Moreover, the clearance capacity is seemingly independent of the powers of resistance of the host, i.e., chronically ill animals remove organisms just as rapidly as normal animals. He also demonstrated something that has been suggested clinically, that all organisms are usually cleared within twenty to thirty minutes.

Despite the apparent inexhaustible power of the reticuloendothelial system to remove bacteria from the circulation in short periods, there is sufficient evidence to indicate that the methods of removal are far from perfect.

With the advent of the sulfonamide compounds it was soon evident that these drugs might be suitable bacteriostatic agents to suppress the inevitable bacteremia that results from dental manipulation.

It is generally agreed that the primary action of sulfanilamide is by means of bacteriostasis, the secondary effect being the disposal of the organisms by means of phagocytosis.[20] This applies, in general, to all sulfonamide compounds, although the exact mechanism of bacteriostasis is still a moot question. However, most authorities agree that sulfanilamide causes a diminution in the rate of growth of the organism. This is borne out by our results, for we found no significant difference between the bacteremia in control subjects and the bacteremia produced when sulfanilamide was present in a concentration of 5 mg. or more per hundred cubic centimeters of blood. It would tend to indicate either that there is no primary lethal effect on the organisms or that the drug is ineffective in vitro, since no effort was made to nullify the effect of the sulfanilamide in culture by means of para-aminobenzoic acid. It was determined, moreover, that the concentration of sulfanilamide in selected cultures, even after seven days, remained unchanged. This suggests that no in vitro effect was exerted, the organisms being able to grow unimpeded. But after ten minutes of circulation in the blood stream the sulfanilamide was able to depress the percentage of bacteria a significant degree, from 33.3 per cent (ten minute result in control group) to 13.3 per cent (ten minute result in sulfanilamide-treated group).

The obtaining of 83.3 per cent positive cultures in 30 cases was found to be highly significant from a statistical analysis point of view. The dif-

19. Reichel, H. A.: Removal of Bacteria from the Blood Stream: Experiments Tending to Determine Rate of Removal of Injected Bacteria in Blood, Proc. Staff Meet., Mayo Clin. **14**:138, 1939.

20. (*a*) Kolmer, J. A.: Progress in Chemotherapy of Bacterial and Other Diseases, Arch. Int. Med. **65**: 671 (April) 1940. (*b*) Mellon, R. R.; Locke, A. P., and Shinn, L. E.: Anti-Enzymatic Nature of Sulphanilamides Bacteriostatic Action, Am. J. M. Sc. **199**: 749, 1940.

ference, however, between 33.3 per cent and 13.3 per cent of positive cultures falls just slightly below the accepted figure of statistical significance. Analysis of our figures according to the chi-square formula yielded a value of 3.36, while the standard figure is 3.84.[21] On further analysis it was found that if we used 36 cases and obtained the same percentage in the results it would be statistically significant.

Since we were not able to obtain sodium polyanetholsulfonate (because of the war), we could not increase our number of cases.

The bacteriostasis appears to have not only a quantitative effect but an immediate qualitative effect as well. Table 5, listing all mixed cultures, shows that there was only 1 mixed culture from the sulfanilamide-treated group while there were 6 mixed cultures from the control group, a difference that is considered significant. It may well be that the quantitative effect is primary. Where there is a heavy shower of organisms, there is a probability of more than one type gaining entry to the blood stream. When this invasion is reduced to only a few organisms through the action of sulfanilamide, there is more likely to be only one type of bacteria.

TABLE 5.—*Mixed Cultures*

Patient No.	Aerobic Immediately After Extraction	Ten Minutes After Extraction	Anaerobic Immediately After Extraction
	Control Group (I)		
1	Viridans streptococci Nonhemolytic streptococci		
4	.....	.....	Viridans streptococci Staph. albus
7	Viridans streptococci Staph. albus	Viridans streptococci Staph. albus	
11	Pneumococci Staph. albus		
16	Viridans streptococci Staph. albus		
	Sulfanilamide-Treated Group (II)		
18	Staph. albus haemolyticus Streptococci (unable to isolate strain)		

Although studies by Long and Bliss[22] Kolmer[20a] and Osgood[23] have shown that sulfanilamide is relatively ineffective against established infection with viridans streptococci, we feel that in view of our results it must be considered to have a definite inhibitory effect, especially during the early phase, when the organism has not as yet established its nidus. Moreover, Spink[12] pointed out that sulfanilamide is bacteriostatic against some strains of the alpha group of streptococci. He also called attention to the contention of some that in the presence of an in vitro anaerobic environment sulfanilamide is markedly bacteriostatic. Our results with the anaerobic cultures confirm that assertion.

Duncan and Faulkner[24] have shown that sulfonamide compounds are powerless to penetrate a blood clot. This emphasizes the importance of having a substance such as sulfanilamide present in the blood stream of susceptible persons to prevent the organism from being established under a protective fibrin layer.

Thomas, France and Reichsman[25] have shown how a small concentration of sulfanilamide can exert sufficient bacteriostatic action to prevent a recurrence of rheumatic fever (associated with beta hemolytic streptococci, for which sulfanilamide has a marked bacteriostasis).

It is readily seen that although the importance of infection of the teeth and gums in the production of systemic infection has long been appreciated, there exists in the literature a great deal of confusion as to the best method of utilizing this knowledge in the prevention of postextraction accidents such as subacute bacterial endocarditis. Even in the absence of rheumatic heart disease, the bacteremia produced by extracting or manipulating teeth can localize in various parts of the body. It may result in an abscess, iritis, or arthritis, or it may cause an old quiescent lesion to flare anew.

It should also be noted that at first there was no scientific basis for suggesting premedication with sulfanilamide when extractions of teeth were contemplated other than the fact that sulfanilamide was known to be bacteriostatic for most of the flora inhabiting the mouth. At the time these experiments were carried out (1941) there were no reports in the literature concerning the effect of drugs in the sulfonamide group on actual postextraction bacteremia. Recently there have been such reports, but in all cases the

21. Mainland, D.: The Treatment of Clinical and Laboratory Data, London, Oliver & Boyd, Ltd., 1938.

22. Long, P. H., and Bliss, E. A.: Clinical Use of Sulfanilamide, Sulfapyridine and Allied Compounds, New York, The Macmillan Company, 1939.

23. Osgood, E. E.; Brownlee, I. E., and Joski, J.: Culture of Human Marrow: Studies of the Relative Effectiveness of Neoarsphenamine, Mapharsen, Sulfanilamide, Sulfapyridine, Sulfathiazole, and Sulfamethylthiazole on Infections with Streptococcus Viridans (Alpha Hemolytic Streptococcus), Am. J. M. Sc. **200**: 596, 1940.

24. Duncan, C. N., and Faulkner, J. M.: Penetration of Blood Clot by Sulfanilamide, Sulfapyridine, Sulfathiazole and Sulfamethylthiazole, Am. J. M. Sc. **200**:492, 1940.

25. Thomas, C. B.; France, R., and Reichsman, F.: The Prophylactic Use of Sulfanilamide, J. A. M. A. **116**:551 (Feb. 15) 1941.

reliability of the results has been negated by the low percentage of positive control cultures.

We feel that the following regimen is advisable in cases of extraction of teeth if the incidence of bacteremia with its subsequent complications is to be eliminated:

1. Physicians should warn patients known to have rheumatic heart disease about the complications that may ensue following extraction of a tooth. Such patients might well be further advised as to the necessity of both medical and dental supervision during all contemplated dental procedures. Dentists, especially exodontists, should attempt to elicit a careful history for rheumatic fever, chorea, scarlet fever, pains in joints, "growing pains," frequent sore throats or any manifestation of the rheumatic diathesis. It cannot be too strongly stressed that such patients should be seen by a physician in consultation with the dentist.

2. Anesthesia. The anesthesia of choice should be that induced with procaine hydrochloride and epinephrine hydrochloride by the infiltration method. Burket and Burn [5c] found that there was a difference in bacteremia results between conduction and infiltration anesthesia, because epinephrine constricted the capillaries and thus prevented the organism from gaining access. In our study we found no difference between infiltration and conduction methods. It is to be noted, however, that we did not use epinephrine. Conduction anesthesia can be considered the same as general anesthesia, since the injection is given distant to the field of extraction. A further substantiation of the use of procaine is the work of Villardo,[26] who demonstrated that procaine inoculated subcutaneously into rabbits increased the bactericidal and phagocytic power of the blood and thus acted both as a systemic and as a local barrier (in combination with epinephrine) to the entrance of organisms into the blood stream.

3. Only one tooth is to be removed at a time, and this with a minimum of rocking and trauma. Multiple extractions are to be condemned. Any manipulative procedure involving either the gums or the teeth is to be avoided, especially in patients known to have rheumatic heart disease.

4. A sulfonamide compound should be administered twenty-four hours prior to the extraction and for twenty-four to forty-eight hours following it in order to obtain the maximum protection. It is doubtful if it is necessary to administer the drug beyond the forty-eight hour period, especially if no more than a single tooth is removed. However, in view of our findings it may not be necessary to administer any of the drug subsequent to extraction. If sulfanilamide is given, sufficient of the drug should be supplied to get a blood level of over 5 mg. per hundred cubic centimeters. This will require between 90 and 110 grains (5.4 and 6.6 gm.) of sulfanilamide in the twenty-four hours prior to the extraction, with the maximum dose given four hours before the extraction. Our dosage was so planned that sulfanilamide was present in the blood in the optimum concentration at the time of extraction and for a few hours thereafter.

26. Villardo, S.: Immunitary Phenomena in Procaine Hydrochloride Anesthesia, Gior. di batteriol. e immunol. **20**:1201, 1938.

SUMMARY

Sulfanilamide exerts no immediate quantitative effect on bacteremia following extraction of teeth but does produce a significant quantitative decrease in organisms that have circulated in the blood stream for ten minutes. The bacteriostasis appears to have not only a delayed quantitative effect but an immediate qualitative effect.

Aerobically, sulfanilamide in vitro exerts no bacteriostatic effect on viridans streptococci but anaerobically has a marked bacteriostasis.

The organisms circulating in the blood stream are few, and a sensitive medium must be used to detect their presence.

INTERCAPILLARY GLOMERULOSCLEROSIS

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The association of intercapillary glomerulosclerosis with a distinct clinical syndrome was first pointed out by Kimmelstiel and Wilson [1] in 1936. They reported 8 cases in which different stages of the renal lesion were present. In all but 1 instance there was a history of diabetes mellitus. The characteristic symptom complex consisted of a history of diabetes mellitus, usually of long standing, widespread edema of renal origin and pronounced albuminuria. Frequently hypertension and renal insufficiency were also present. The renal lesion was characterized by focal hyalinization of the intercapillary connective tissue and was named intercapillary glomerulosclerosis.

The concomitant occurrence of these glomerular lesions with all or most of the manifestations of the typical syndrome was subsequently confirmed by Murakami,[2] Anson,[3] Derow, Altschule and Schlesinger,[4] Newburger and Peter,[5] Porter and Walker,[6] Siegal and Allen,[7] Herbut [8] and Allen.[9] The existence of intercapillary glomerulosclerosis without a typical symptom complex was noted by Anson,[3] Porter and Walker,[6] Siegel and Allen,[7] Herbut,[8] Horn and Smetana [10] and Bell.[11] Horn and Smetana [10] found that the characteristic clinical features were lacking in many diabetic patients with glomerular lesions. Bell [11] stated that there are no definite clinical features by which diabetes with intercapillary glomerulosclerosis can be distinguished from diabetes without this lesion.

In their original publication Kimmelstiel and Wilson [1] said that the renal lesion was of infrequent occurrence. Other reports disclose, however, that intercapillary glomerulosclerosis is not uncommon in diabetes. The majority indicate that it is rare in persons without diabetes. The publications of Siegal and Allen,[7] Herbut [8] and Allen [9] emphasize its specificity and indicate that it is a useful criterion for the postmortem diagnosis of diabetes mellitus. Horn and Smetana,[10] however, described similar glomerular lesions in many nondiabetic persons. In that study intercapillary glomerulosclerosis occurred in 22.9 per cent of diabetic patients, in 25.4 per cent of patients with arteriolar nephrosclerosis, in 11.1 per cent of patients with generalized arteriolar sclerosis without diabetes or renal disease and in 6.5 per cent of patients with glomerulonephritis. They concluded that although extreme degrees of this condition are always associated with diabetes the typical focal glomerular hyalinization is not constantly associated with the clinical syndrome which includes diabetes mellitus.

The present study was undertaken in order to determine the incidence of intercapillary glomerulosclerosis and to correlate its occurrence and development with distinctive clinical manifestations. For this purpose the clinical records, autopsy protocols and microscopic sections of 332 patients were examined.

METHODS

The patients with diabetes mellitus were divided into three groups on the basis of the degree of their disease. The diabetes was considered to be: mild, if it was

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3. Anson, L. J.: Intercapillary Glomerulosclerosis, South. M. J. **31**:1272, 1938.

4. Derow, H. A.; Altschule, M. D., and Schlesinger, M. J.: Syndrome of Diabetes Mellitus, Hypertension and Nephrosis: Clinical and Pathological Study of Case, New England J. Med. **221**:1012, 1939.

5. Newburger, R. A., and Peters, J. P.: Intercapillary Glomerulosclerosis: A Syndrome of Diabetes, Hypertension and Albuminuria, Arch. Int. Med. **64**: 1252 (Dec.) 1939.

6. Porter, W. B., and Walker, H.: The Clinical Syndrome Associated with Intercapillary Glomerulosclerosis, J. A. M. A. **116**:459 (Feb. 8) 1941.

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controlled with 10 units or less of insulin per twenty-four hours; moderate, if 11 to 25 units per twenty-four hours was needed, and severe, when more than 25 units per twenty-four hours was required.

The existence of hypertension was determined from the blood pressure readings taken during hospitalization and from the postmortem examination of the heart. Systolic hypertension was considered to have been present if the systolic blood pressure was 150 mm. of mercury or more. When the diastolic pressure was 95 mm. of mercury or more, diastolic hypertension was considered to have existed. When the existence of hypertension was doubtful because of inadequate blood pressure readings, particularly in patients who died of cardiac failure consequent to myocardial infarction, the presence or absence of cardiac hypertrophy was the deciding factor. In these instances, when cardiac hypertrophy existed, as determined by gross and microscopic examination, without significant anatomic disease of cardiac valves or congenital cardiovascular anomalies, systolic and diastolic hypertension were considered to have been present during life.

Examination of the clinical records furnished data concerning the presence or absence of diabetic acidosis. It was considered to have been present with or without coma when the urine contained acetone and diacetic acid.

The complete nephrotic syndrome includes pronounced albuminuria, anasarca, lowered plasma proteins and hypercholesteremia. In our series of cases there were many instances in which the blood cholesterol level was not determined. For this reason and because other reports include cases with incomplete nephrotic syndromes, hypercholesteremia was not considered essential for the establishment of this symptom complex. In all instances, however, those considered to have this syndrome had 3 to 4 plus albuminuria, generalized edema involving the face and body cavities as well as dependent parts of the body and total plasma proteins below 6.00 mg. per hundred cubic centimeters.

Patients were considered to have uremia when there was a considerable elevation of blood urea nitrogen (above 60 mg. per hundred cubic centimeters) and blood creatinine (above 3 mg. per hundred cubic centimeters). In most instances uremic patients became comatose before death occurred. In some cases cardiac failure was also present, so that it was impossible to determine whether or not renal disease alone was sufficient to cause the uremia.

In a few instances the tissues were fixed in Zenker's solution prepared with formaldehyde (20:1), but in the majority they were fixed in a 4 per cent solution of formaldehyde. In all cases included in the series not less than two sections of each kidney and two sections from the tail of the pancreas were examined. These measured roughly 2 by 1.5 cm. The following stains were used: hematoxylin and eosin; Mallory-Heidenhain azocarmine; Wilder's silver; Best's carmine; Van Gieson's stain; sudan IV; osmic acid; nile blue sulfate; congo red, and crystal violet.

The kidneys were graded as to the degree of development of intercapillary glomerulosclerosis and renal vascular sclerosis. The arbitrary classification (1 to 4 plus) of the vascular changes in the kidneys was determined by estimating the degree and diffuseness of intimal fibrosis and proliferation of arteries and arterioles. In this grouping the sclerosis of the arterioles was given more weight than that of the arteries. Thus kidneys showing 2 plus arteriosclerosis and 3 plus arteriolar sclerosis were graded as having 3 plus involvement. On the other hand, those with 3 plus sclerosis of arteries and 2 plus sclerosis of arterioles were assigned to the 2 plus group.

The pancreases were also graded in accordance with the amount of hyalinization of the islets of Langerhans. The basis of this classification was an estimation of the number of islets showing hyalinization and the quantitative changes in individual islets.

MORPHOLOGY OF THE GLOMERULAR LESION

The typical lesion (fig. 1) of intercapillary glomerulosclerosis, described originally by Kimmelstiel and Wilson, is usually spherical and occasionally oval. It varies from 20 to 110 microns in maximum diameter and is made up of faintly acidophilic acellular hyalinized tissue. With low magnification it appears homogeneous, but with high magnification small vacuoles and with the Wilder silver stain circumferential lamination are frequently evident. The hyaline material does not have the specific staining properties of amyloid. It stains either red or blue with the Mallory-Heidenhain azocarmine and pale yellow with the Van Gieson stain. Small droplets of fat are not uncommonly present in the hyaline material. These are, however, no more numerous and no larger than the lipid droplets which occur in the kidneys of nondiabetic persons and of persons with diabetes without typical lesions. Consequently, it is impossible to attach much differential significance to these lipid deposits. At the periphery of the lesion there are usually one or more concentric layers of flattened cells, presumably endothelial. The involved glomeruli sometimes are small but more frequently are of normal or larger size.

The degree of development of the lesion varies considerably. There may be many typical spherical lesions in a single glomerulus (fig. 2), or there may be only one in several sections of the kidneys. In the kidneys of diabetic patients with typical spherical lesions there is also focal fibrosis of nearly all of the glomeruli. The association of these glomerular changes led to the recognition of a lesser degree (1 plus) of development of the lesion (fig. 3), in which there is focal fibrosis of the majority of glomeruli without spherical hyaline masses. There are in many cases definite transitions from this focal glomerular fibrosis and the circumscribed hyaline masses (figs. 3, 4 and 5).

Since sclerosis means overgrowth of connective tissue, it seemed logical to interpret the glomerular fibrosis as a stage of intercapillary glomerulosclerosis less severe than those previously recognized. However, in order to determine the validity of these observations sections of the kidneys

showing this degree of the lesion were mixed with others from an equal number of patients without diabetes. It was then possible to distinguish objectively those with 1 plus intercapillary glomerulosclerosis from those without diabetes and without the spherical glomerular lesion.

This stage of development of the condition is not described in other reports. Its recognition and inclusion in part account for the high incidence of intercapillary glomerulosclerosis in our patients. This degree of glomerular involvement (1 plus) occurred in 24.1 per cent (19 of 79) of

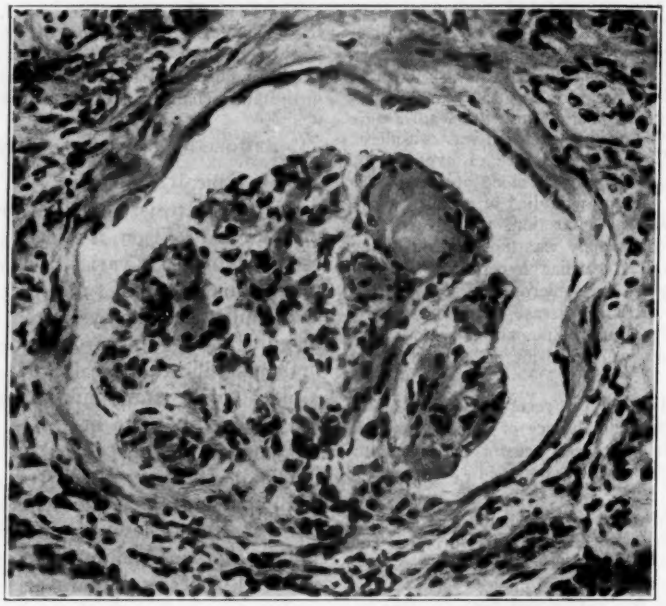

Fig. 1.—Photomicrograph showing a glomerulus with a typical circular hyaline mass with flattened endothelial cells at the periphery. Hematoxylin and eosin; × 312.

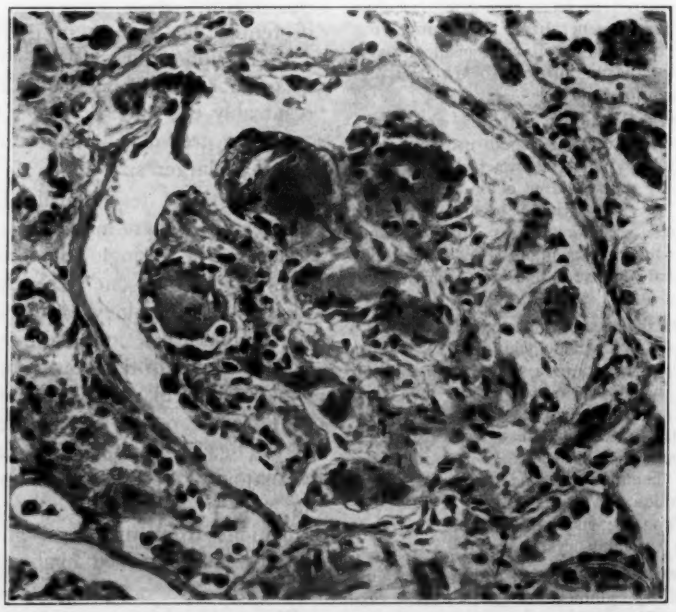

Fig. 2.—Photomicrograph illustrating a pronounced degree of development of intercapillary glomerulosclerosis. There are several circular hyaline masses in a single glomerulus. Hematoxylin and eosin; × 312.

the diabetic patients with intercapillary glomerulosclerosis. In these patients nearly all of the glomeruli in both kidneys showed an approximately equal degree of focal fibrosis but no characteristic spherical hyaline masses. In the absence of diabetes, fibrosis also occurs in some of the glomeruli of kidneys that are the seat of arterial and arteriolar nephrosclerosis, chronic pyelonephritis or chronic glomerulonephritis. In these, however, the absence of typical intercapillary glomerulosclerosis can be excluded by the variation in the degree of glomerular fibrosis,

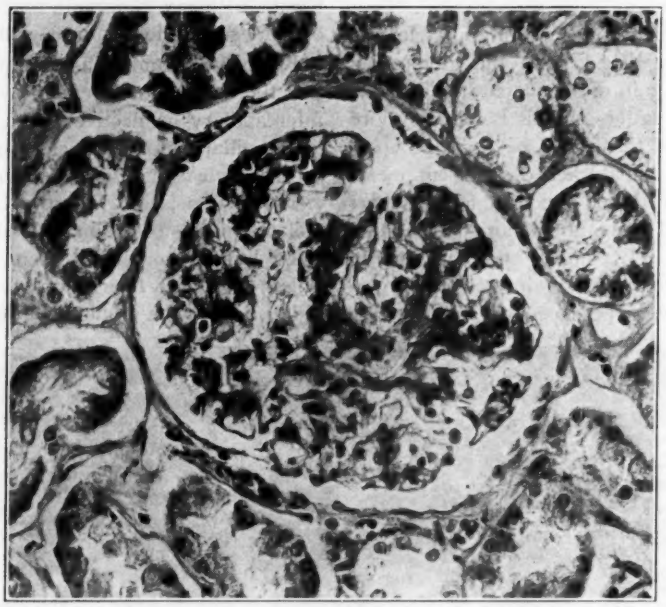

Fig. 3.—Figures 3, 4 and 5 show various degrees of development of intercapillary glomerulosclerosis. In figure 3 there is focal fibrosis of the glomerulus. Hematoxylin and eosin; × 312.

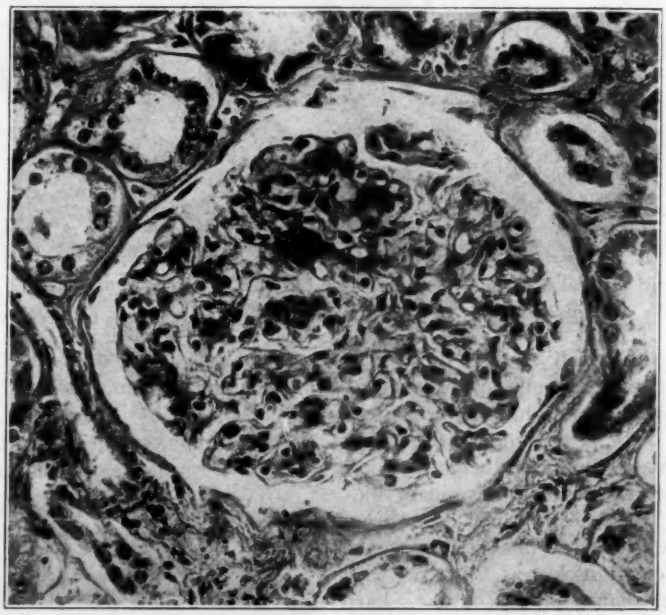

Fig. 4.—Focal fibrosis with small circular masses and centrally placed nuclei. Hematoxylin and eosin; × 312.

the presence of many normal glomeruli, the occurrence of adhesions between glomerular tufts and capsules of Bowman and a variable degree of atrophy of tufts.

All stages of intercapillary glomerulosclerosis are readily distinguished from the glomerular lesions which occur in disseminated lupus erythematosus. In the latter, fibrosis is usually more diffuse, with the formation of "wire loops." Characteristically the thickened basement membrane of the "wire loop" lesion at least in part stains pink or red with the Mallory-Heidenhain azocarmine stain. In similar preparations of kidneys that are the seat of intercapillary glomerulosclerosis, with the exception of the spherical hyaline masses which are in themselves characteristic, the proliferated glomerular connective tissue is dark blue. The foci of necrosis which sometimes occur in the kidneys of patients dying of disseminated lupus erythematosus are not a part of the picture of intercapillary glomerulosclerosis. These necrotic foci differ from the characteristic spherical hyaline masses of intercapillary glomerulosclerosis in that they lack the spherical shape, regular outline, sharp circumscription, vacuolation and peripherally placed nuclei of the latter.

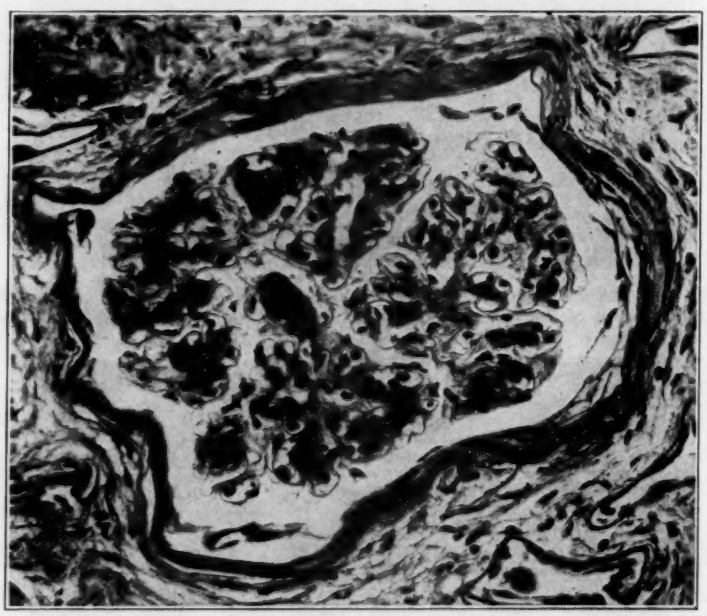

Fig. 5.—More marked focal fibrosis of the glomerular tuft than is seen in figures 3 and 4, and a small typical circular hyaline mass. Hematoxylin and eosin; × 312.

In this series the kidneys were graded as to the degree of development of intercapillary glomerulosclerosis. With 1 plus glomerulosclerosis there was focal fibrosis of the majority of the glomeruli but no spherical hyalinized lesions; with 2 plus, only an occasional spherical mass in four or more sections; with 3 plus, spherical lesions in many glomeruli, and with 4 plus, one or more such lesions in nearly all of the glomeruli.

Kimmelstiel and Wilson expressed the belief that the lesions develop from hyalinization of the intercapillary connective tissue. Thus the term intercapillary glomerulosclerosis, which has acquired wide usage, was affixed to the lesions. Serial sections, however, reveal the lesions to be isolated masses of hyalin with small endothelium-lined lumens containing blood cells near their poles (fig. 6). The lesions are therefore considered to be the result of profound focal thickening and hyalinization of the capillary loops or their basement membranes.

INCIDENCE OF INTERCAPILLARY GLOMERULOSCLEROSIS

Table 1 indicates the incidence of the glomerular lesions in diabetic patients as reported by different authors.

In our series of cases of diabetes (table 2) intercapillary glomerulosclerosis was common, occurring in 63.7 per cent (79 of 124) of the patients. As previously indicated, the high incidence is in part due to the recognition of a slight degree of development of the lesion which has not been included in other publications.

Tables 3 and 4 show the degree of development and the incidence of intercapillary glomerulosclerosis in males and in females with diabetes mellitus. There is no statistically significant difference in its occurrence in the two sexes. In both sexes the glomerular lesions are most numerous and most severe in the seventh decade. The occurrence of typical lesions in a 16 year old white girl who had had severe diabetes for ten years is worthy of note. Her clinical course was characteristic of so-called juvenile diabetes. This case is particularly significant because glomerular changes were not associated with renal arteriolar sclerosis.

As indicated in table 2, intercapillary glomerulosclerosis is uncommon in nondiabetic persons, typical glomerular lesions occurring in only 5 instances in which a history of diabetes could not be established. In one instance there was associated chronic pyelonephritis of moderate

TABLE 1.—*Incidence of Intercapillary Glomerulosclerosis in Diabetes Mellitus*

Author	Number of Persons with Diabetes	Intercapillary Glomerulosclerosis	
		Number of Patients	Per Cent
Siegal and Allen [7]	105	35	33.3
Horn and Smetana [10]	144	33	22.9
Bell [11]	460	115	20.5
Laipply, Eitzen and Dutra	124	79	63.7

TABLE 3.—*Intercapillary Glomerulosclerosis in Males*

Age Group	Number of Patients	Present	Degree of Development				Absent
			1+	2+	3+	4+	
20	1	0	..	..	..	..	1
30-39	0	0	..	..	..	..	0
40-49	8	3	1	2	..	..	5
50-59	13	8	..	4	4	..	5
60-69	27	19	5	8	3	3	8
70-79	6	2	1	1	..	..	4
80-89	2	1	..	1	..	..	1
Total	57	33	7	16	7	3	24
Per cent.		57.9	12.3	28.1	12.3	5.3	42.2

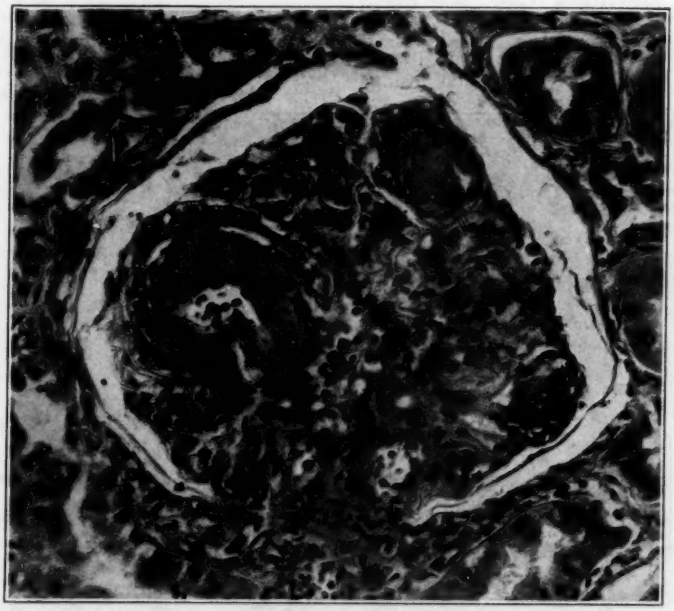

Fig. 6.—The glomerulus contains three typical circular areas of hyalinization. In the center of the largest one there is an endothelium-lined space which contains blood cells. Hematoxylin and eosin; ×312.

TABLE 2.—*Intercapillary Glomerulosclerosis in Diabetes and Renal Disease*

	Total Number of Cases	With Intercapillary Glomerulosclerosis	
		Number	Per Cent
Diabetes	124	79	63.7
Arterial and arteriolar nephrosclerosis	124	1	0.8
Chronic pyelonephritis	60	1	1.7
Subacute and chronic glomerulonephritis	24	3	12.5

TABLE 4.—*Intercapillary Glomerulosclerosis in Females*

Age Group	No. of Patients	Present	Degree of Development				Absent
			1+	2+	3+	4+	
16	1	1	1	..	..	..	0
30-39	2	1	..	1	..	..	1
40-49	10	7	3	2	1	1	3
50-59	15	9	5	1	2	1	6
60-69	25	19	3	6	3	7	6
70-79	10	7	..	3	3	1	3
80-89	4	2	..	2	..	..	2
Total	67	46	12	15	9	10	21
Per cent		68.6	17.9	22.4	13.4	14.9	31.3

degree, in another marked arterial and arteriolar nephrosclerosis, in 1 subacute glomerulonephritis and in 2 others chronic glomerulonephritis (fig. 7). In all of these there were characteristic spherical masses of hyaline in the glomeruli. In 4 instances the degree of development of intercapillary glomerulosclerosis was 2 plus, and in 1 it was 3 plus. The patient with glomerular changes graded as 3 plus had chronic glomerulonephritis and a few months prior to death had glycosuria. The possibility of diabetes mellitus could not, however, be established from the clinical data available, and consequently the case is not included in the series of cases of diabetes.

This infrequent occurrence of the typical glomerular changes in nondiabetic persons supports the work of Siegal and Allen,[7] who found them in only 1 of a series of 200 patients without diabetes. In Allen's paper[9] reference is made to 2 other cases in which such lesions were subsequently discovered by chance in nondiabetic persons who were not included in their earlier series. In these cases the possibility of incipient or latent diabetes cannot be excluded.

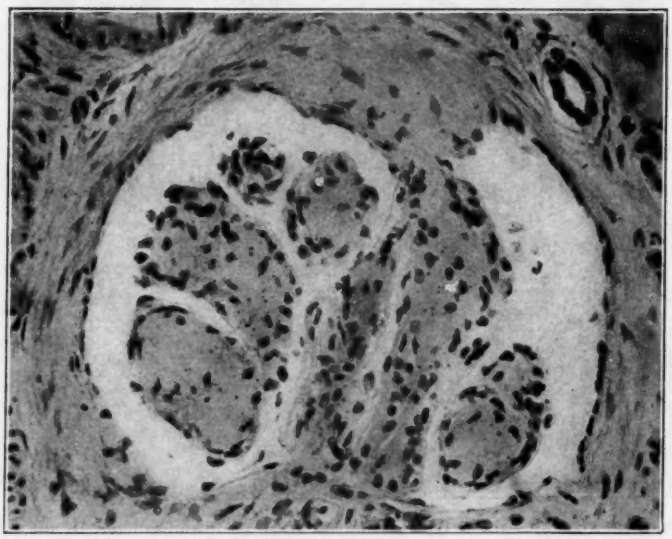

Fig. 7.—Photomicrograph showing a lobulated glomerulus with typical circular hyaline masses, fibrosis, and adhesion between the glomerulus and the thickened capsule of Bowman. The patient was a nondiabetic person with chronic glomerulonephritis. Hematoxylin and eosin; × 312.

RELATION TO DIABETES MELLITUS AND TO PANCREATIC ISLET HYALINIZATION

The high incidence of intercapillary glomerulosclerosis in diabetes and its infrequent occurrence in the absence of diabetes suggest a causal relation. There is, however, no significant correlation between the presence or degree of development of the glomerular lesions and the degree (table 5) or duration (table 6) of the diabetes. Similar tabulations reveal an absence of correlation between the degree of hyalinization of the islets of Langerhans and the degree or duration of the diabetes. The histories reveal no demonstrable relation between the specific treatment for diabetes and the development of the renal or pancreatic lesions.

In 2 of the diabetic patients with intercapillary glomerulosclerosis the material from the pan-

TABLE 5.—*Relation of Intercapillary Glomerulosclerosis to Degree of Diabetes*

Degree of Diabetes	No. of Patients	Degree of Intercapillary Glomerulosclerosis				
		0	1+	2+	3+	4+
Mild	54	19 35.2%	8 14.8%	17 31.5%	6 11.1%	4 7.4%
Moderate	40	16 40.0%	6 15.0%	6 15.0%	7 17.5%	5 12.5%
Severe	39	10 25.6%	5 12.8%	7 17.9%	3 7.7%	4 10.3%
Unknown	1	..	..	1	..	..

TABLE 6.—*Relation of Degree of Intercapillary Glomerulosclerosis to Duration of Diabetes*

Duration	No. of Patients	Degree of Intercapillary Glomerulosclerosis				
		0	1+	2+	3+	4+
Less than 1 year	23	9 39.1%	9 39.1%	5 21.7%	0	0
1 to 4 years	28	15 53.6%	3 10.7%	4 14.3%	3 10.7%	3 10.7%
5 to 9 years	34	15 44.1%	4 11.8%	6 17.6%	5 14.7%	4 11.8%
10 to 14 years	19	2 10.5%	2 10.5%	10 52.6%	4 21.1%	1 5.3%
15 years or more	16	2 12.5%	1 6.3%	5 31.3%	4 25.0%	4 25.0%
Unknown	4	2	..	1	..	1

creas which was available for microscopic examination was considered inadequate, and a comparison of the renal and pancreatic lesions could not be made. Table 7 shows the relative incidence of intercapillary glomerulosclerosis and hyalinization of islets of Langerhans in the diabetic patients from whom adequate material was obtainable. Renal lesions and hyalinization of the islets occurred in 63.1 per cent (77 of 122). Thus, intercapillary glomerulosclerosis was just as common as hyalinization of the pancreatic islets in patients subjected to autopsy who had diabetes mellitus.

The lack of correlation between the degree of the glomerular changes and the degree of hyalinization of the pancreatic islets is indicated in table 8. Thus, in the patients with 4 plus intercapillary

TABLE 7.—*Intercapillary Glomerulosclerosis and Hyalinization of Islets of Langerhans*

Age Group	No. of Patients	Intercapillary Glomerulosclerosis		Hyalinization of Islets of Langerhans	
		Number	Per Cent	Number	Per Cent
10	1	1	....	0	....
20	1	0	....	0	....
30-39	2	1	....	2	....
40-49	18	10	55.6	11	61.1
50-59	26	15	57.7	15	57.7
60-69	52	38	73.1	35	67.3
70-79	16	9	56.3	8	50.0
80-89	6	3	50.0	6	100.0
Total	122	77	63.1	77	63.1

TABLE 8.—*Relation of Renal and Pancreatic Lesions*

Degree of Intercapillary Glomerulosclerosis	No. of Patients	Degree of Hyalinization of Islets of Langerhans					
		0	1+	2+	3+	4+	Average
0	45	21	9	9	3	3	1.07
1+	18	2	7	5	3	1	1.67
2+	31	8	15	3	4	1	1.19
3+	15	8	4	2	0	1	0.80
4+	13	6	5	1	1	0	0.77

glomerulosclerosis the average degree of hyalinization of the islets was less than in those without typical glomerular lesions.

Frozen sections of pancreases showing 3 to 4 plus hyalinization of the islets of Langerhans were stained with crystal violet. In this way the hyaline material in the islets was shown to have the specific staining property of amyloid. The fact was previously reported by Arey [12] and Ahronheim.[13] The hyalin in the glomerular lesions, on the other hand, does not react positively with stains for amyloid.

It would seem, therefore, that diabetes mellitus is not ordinarily the sole etiologic factor in the development of the characteristic renal lesion which commonly accompanies it. Other facts brought out in this study fail, however, to disclose any other causative agent. In addition, the occurrence of intercapillary glomerulosclerosis in the 16 year old diabetic girl in the absence of significant renal arteriolar sclerosis and hypertension tends to minimize the possibility of the existence of any dependent factor.

In an endeavor to discover some additional causative agent, the kidneys of 3 patients with chronic lipid nephrosis were examined. In none of these were there characteristic spherical hyaline glomerular masses, but in all of them the glomeruli were as large as or larger than normal and showed a moderate degree of fibrosis which was focal in distribution. These changes are not unlike those seen in slight (1 plus) intercapillary glomerulosclerosis. This suggests the possibility that the abnormal metabolism which exists in diabetic persons and in patients with chronic lipid nephrosis may be important in the development of intercapillary glomerulosclerosis.

RELATION TO ARTERIAL AND ARTERIOLAR SCLEROSIS OF THE KIDNEYS

The greater frequency and greater severity of arterial and arteriolar sclerosis in patients with than in those without diabetes is well known. For this reason the possibility of renal vascular sclerosis being a causative factor of intercapillary glomerulosclerosis has received serious consideration. Thus, Newberger and Peters [5] stated that "the pathogenesis of this condition appears to depend on severe and extensive arterial and arteriolar degeneration, associated with and perhaps resulting in diabetes mellitus, hypertension and renal damage." The majority of papers on this subject have called attention to the extremely high incidence of renal arterial and arteriolar sclerosis in patients with intercapillary glomerulosclerosis.

Table 9 shows the degree of renal arterial and arteriolar sclerosis associated with varying grades of the characteristic glomerular changes in our series of diabetic patients. All of these patients had some degree of sclerosis of renal arteries, arterioles or both. In most instances

12. Arey, J. B.: Nature of the Hyaline Changes in Islands of Langerhans in Diabetes Mellitus, Arch. Path. **36**:32 (July) 1943.

13. Ahronheim, J. H.: The Nature of the Hyaline Material in the Pancreatic Islands in Diabetes Mellitus, Am. J. Path. **19**:873, 1943.

those with the greatest degree of intercapillary glomerulosclerosis had the most pronounced vascular changes. It should be noted (tables 3 and 4), however, that extreme degrees of intercapillary glomerulosclerosis occurred nearly always in patients over 50 years of age. Diabetic patients of this age would be expected to have more or less severe renal arterial and arteriolar sclerosis. Thus, the possibility of coincidental occurrence of the two conditions exists. A lack of causal relationship between the two is also suggested by the occurrence of intercapillary glomerulosclerosis in persons with diabetes without severe renal vascular sclerosis and by its infrequency in nondiabetic persons with significant vascular changes in the kidneys.

TABLE 9.—*Relation of Intercapillary Glomerulosclerosis to Arterial and Arteriolar Sclerosis of the Kidneys*

Degree of Intercapillary Glomerulosclerosis	No. of Patients	Degree of Renal Vascular Sclerosis			
		1+	2+	3+	4+
0	45	18 40.0%	16 35.6%	9 20.0%	2 4.4%
1+	19	4 21.1%	7 36.8%	6 31.6%	2 10.5%
2+	31	1 3.2%	11 35.5%	11 35.5%	8 25.8%
3+	16	0	4 25.0%	6 37.5%	6 37.5%
4+	13	0	1 7.7%	6 46.2%	6 46.2%

In the series of diabetic patients, 5 of those listed as having 1 plus renal vascular sclerosis had no demonstrable arteriolar involvement. One of these, a 16 year old white girl who had had severe diabetes for ten years, showed slight (1 plus) intercapillary glomerulosclerosis.

The three control groups of nondiabetic persons all included patients with renal vascular sclerosis. One of these consisted of 124 patients with arterial and arteriolar sclerosis of the kidneys. The age distribution of this series was the same as that of the diabetic patients. Systolic and diastolic hypertension had been present in 89 and absent in 35 patients. The degree of renal vascular sclerosis was: in 15 cases, 4 plus; in 32, 3 plus; in 41, 2 plus, and in 36, 1 plus. Only 1 of these patients had intercapillary glomerulosclerosis. This patient had had systolic and diastolic hypertension and had moderate (2 plus) sclerosis of the renal vessels.

The second control group consisted of 60 nondiabetic persons with chronic pyelonephritis. The renal arterial and arteriolar sclerosis was graded as 4 plus in 33 of these patients, as 3 plus in 14, as 2 plus in 11 and as 1 plus in 2. Fifty-two had had hypertension. In only 1 patient was intercapillary glomerulosclerosis present. This patient had also had systolic and diastolic hypertension, and the degree of renal vascular sclerosis was 4 plus.

The third control series of nondiabetic persons was made up of 24 patients with subacute or chronic glomerulonephritis. All of the kidneys of these patients showed arterial and arteriolar sclerosis. In 3 patients it was 4 plus; in 8, 3 plus; in 6, 2 plus, and in 7, 1 plus. Intercapillary glomerulosclerosis occurred in 3 of this series. In 1 patient the glomeurlonephritis was subacute and the renal vascular sclerosis was 1 plus; in the 2 others the glomerulonephritis was chronic and the renal arterial and arteriolar sclerosis was 3 and 4 plus. Systolic and diastolic hypertension had been present in these 3 and in 15 others without intercapillary glomerulosclerosis.

It is thus evident that renal arterial and arteriolar sclerosis is not the sole factor in the development of intercapillary glomerulosclerosis. That it is of basic importance seems unlikely, but its being a contributing factor cannot be excluded.

RELATION TO HYPERTENSION

Although present more often than not in persons with intercapillary glomerulosclerosis, hypertension is not a necessary part of the clinical picture (table 10).

TABLE 10.—*Relation of Intercapillary Glomerulosclerosis to Hypertension*

Degree of Intercapillary Glomerulosclerosis	No. of Patients	Hypertension			
		Systolic and Diastolic	Systolic	Absent	Unknown
0	45	25 55.6%	2 4.4%	18 40.0%	0
1+	19	12 63.2%	0	6 31.6%	1 5.3%
2+	31	19 61.3%	1 3.3%	9 29.1%	2 6.5%
3+	16	11 68.8%	1 6.3%	1 6.3%	3 18.8%
4+	13	9 69.2%	1 7.7%	3 23.1%	0

In our series, intercapillary glomerulosclerosis was present in 67.1 per cent (51 of 76) of diabetic patients with systolic and diastolic hypertension. Systolic and diastolic hypertension was present in 64.6 per cent (51 of 79) of diabetic patients with intercapillary glomerulosclerosis. There was no demonstrable relation between the stage of the glomerular lesion and the presence or absence of hypertension.

The hypertension when present is in all probability associated with the arterial and arteriolar nephrosclerosis which is commonly present in diabetes mellitus.

RELATION TO ALBUMINURIA

The relation between intercapillary glomerulosclerosis and albuminuria is shown in table 11. Albuminuria was common in patients with typical glomerular lesions, being present in 81 per cent (64 of 79) of diabetic patients with intercapillary glomerulosclerosis. There was no albuminuria in only 7.6 per cent (6 of 79) of diabetic patients with the glomerular lesions. In practically all instances in which the glomerular lesions were severe albumin was present in the urine. Albuminuria did, however, occur in many (71.1 per cent, or 32 of 45) diabetic patients in the absence of intercapillary glomerulosclerosis.

The exact cause of the albuminuria was difficult to determine. In many instances the renal arterial and arteriolar nephrosclerosis was of sufficient magnitude to cause albuminuria. In other cases it was at least in part due to congestive cardiac failure and diabetic acidosis. Cardiac failure occurred in 32.8 per cent (21 of 64) of the patients; diabetic acidosis was present in 56.3 per cent (36 of 64) of the diabetic patients with albuminuria and intercapillary glomerulosclerosis.

TABLE 11.—*Relation of Albuminuria to Intercapillary Glomerulosclerosis*

Degree of Intercapillary Glomerulosclerosis	No. of Patients	Degree of Albuminuria					
		0	1+	2+	3+	4+	Unknown
0	45	11 24.4%	16 35.5%	6 13.3%	8 17.8%	2 4.4%	2 4.4%
1+	19	1 5.3%	7 36.8%	2 10.5%	2 10.5%	4 21.1%	3 15.8%
2+	31	3 9.7%	14 45.2%	6 19.4%	5 16.1%	0	3 9.7%
3+	16	2 12.5%	4 25.0%	2 12.5%	4 25.0%	3 18.8%	1 6.3%
4+	13	0	2 15.4%	3 23.1%	7 53.8%	1 7.7%	0

RELATION TO THE NEPHROTIC SYNDROME AND UREMIA

A typical nephrotic syndrome was present in only 6.3 per cent (5 of 79) of the patients with diabetes mellitus and intercapillary glomerulosclerosis. Although it is not common, the syndrome when present is characteristic, including pronounced albuminuria, extensive edema, low plasma proteins and in some instances elevated blood cholesterol. In 3 of the patients having this symptom complex the degree of intercapillary glomerulosclerosis was 4 plus; in 1 it was 3 plus, and in the fifth it was 1 plus. Thus, in most of the diabetic patients who had a nephrotic syndrome the glomerular changes were severe. The nephrotic syndrome did not occur in any of the diabetic patients in the absence of intercapillary glomerulosclerosis.

The patient with chronic glomerulonephritis and 3 plus intercapillary glomerulosclerosis listed as a nondiabetic person also had a typical nephrotic syndrome. In the case of this patient, because of the existence of glycosuria the possibility of diabetes could not be positively excluded.

Uremia occurred more often in the diabetic patients with than in those without intercapillary glomerulosclerosis. It was present in 17.7 per cent (14 of 79) of those with the characteristic renal lesions and in 11.1 per cent (5 of 45) of those without the characteristic glomerular changes. There was, however, no statistically significant difference in incidence.

SPECIFICITY OF INTERCAPILLARY GLOMERULOSCLEROSIS

The relative incidence of intercapillary glomerulosclerosis and hyalinization of the islets of Langerhans in nondiabetic persons is shown in table 12. Glomerular lesions occurred in only 2.4 per cent, while slight hyalinization of the pancreatic islets was present in 13 per cent. In persons with diabetes, on the other hand (table 7), characteristic glomerular fibrosis and hyalinization were present as frequently as hyalinization of islets, occurring in the majority (63.1 per cent). It is thus obvious that intercapillary glomerulosclerosis is a more specific anatomic lesion than hyalinization of the islets of Langerhans.

TABLE 12.—*Incidence of Renal and Pancreatic Lesions in Nondiabetic Patients*

Total No. of Patients	Intercapillary Glomerulosclerosis		Hyalinization of Islets of Langerhans	
	Number	Per Cent	Number	Per Cent
208	5	2.4	27	13.0

The postmortem diagnosis of diabetes mellitus has in the past been dependent on demonstration of hyalinization of the islets of Langerhans in the pancreas and infiltration of the cells of the loops of Henle with glycogen. Anatomic lesions are, however, not constantly present, and their evaluation is sometimes difficult. Thus there is usually no demonstrable glycogen in the renal tubule cells, because it rapidly disappears from the tissues after death and it is frequently absent in insulin-treated patients. Likewise, hyalinization of the pancreatic islets is not constant and when present is not pathognomonic of diabetes.

It is thus evident, because of its high incidence and relative specificity, that intercapillary glomerulosclerosis is the most reliable criterion available at the present time for the postmortem diagnosis of diabetes mellitus.

CONCLUSIONS

1. At autopsy intercapillary glomerulosclerosis is a common lesion in diabetic persons and an uncommon one in nondiabetic persons.

2. It is more specific than hyalinization of the pancreatic islets as an indication of diabetes mellitus.

3. Because of its high incidence and clearcut character, it is at present the most reliable criterion available for the postmortem diagnosis of diabetes mellitus.

4. There is no demonstrable relation between the degree of its development and the duration or degree of the diabetes.

5. It is not necessarily associated with hypertension, albuminuria, renal arterial and arteriolar sclerosis, uremia or the nephrotic syndrome.

6. The nephrotic syndrome is not a common accompaniment. It occurred in only 6.3 per cent of the diabetic patients with intercapillary glomerulosclerosis.

MENINGOCOCCIC MENINGITIS IN SANTIAGO, CHILE, 1941 TO 1943

AN EPIDEMIC OF 4,464 CASES

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An epidemic of meningococcic meningitis started in the port of Valparaiso, Chile, in June 1941 and extended from there until it reached Santiago, the capital, three to four months later. However, it was during the year 1942 that the largest number of cases was observed. Valparaiso is surrounded by hills which are close to the sea. Most of its 200,000 inhabitants live on these hills, in poor hygienic conditions. Santiago, the capital of the most pleasant and civilized country in South America, as John Gunther wrote in his book "Inside Latin America," is a city of 1,290,000 inhabitants and is located about 80 miles (129 kilometers) southeast of Valparaiso, close to the first peaks of the Andes range.

The total number of cases recorded in Santiago from September 1941 to July 3, 1943 was 4,464. The average population of Santiago for these years was 1,290,000, and on this basis there has occurred approximately 1 case per 300 inhabitants. The majority of the cases appeared in the crowded poor neighborhoods. Later the outbreak extended to the rest of the country, but without reaching the importance it had in the capital and in Valparaiso. One of the reasons that has been given for the spreading of the epidemic is that many citizens had to move from one area of the country to another to join their regiments because of the compulsory military service. However, these small outbreaks were readily controlled.

What were the possible causes of the epidemic? They are difficult to point out. From 1920 to 1937 the total number of cases for the entire country was 60, with a high fatality rate, as was the rule when serum was the only treatment available.

In the year 1938 there was a small outbreak in a school of navy pilots on the island of La Quiriquina, off the south coast of Chile. There is a continuous movement of ships between this island and Valparaiso, and it could be the possible focus of carriers.

The year 1942 was the coldest and most rainy of the last sixty years. During the years 1940 and 1941 a great number of German and Spanish refugees reached the country, persons who might have been carriers of new strains of meningococci.

To the sanitary authorities of Chile the variations of the weather seem to be the most important contributing factor, but this by no means represents the prime cause of the epidemic. The epidemiologic factors which predispose to the occurrence of meningococcic meningitis, namely, fatigue, poor nutrition, overcrowding and poor hygienic conditions, have been present in this country for many years. Chile is a young country, with few economic resources and a low standard of living, though at present it is in the process of active development. The disease had been endemic in Chile for years; hence the most important determining cause, the presence of the meningococcus, was not, by itself, enough to explain the sudden change from endemic to epidemic proportions. With epidemic communicable diseases it is frequently difficult to find the contributing cause that determines their behavior because of the multiplicity of factors which are operative in any community.

It has been stated that one may speak of an epidemic of meningococcic meningitis when there is at least 1 case for every 800 inhabitants. On this basis we can consider the Chilean epidemic, with its 1 case per 300 population, as one of the most serious reported in the literature. The greatest number of cases was observed from the middle of winter to the end of spring, that is, from July to November.

The data were compiled to show morbidity, mortality and fatality rates. There were 35 cases and 6 deaths per ten thousand of the population. The total number of deaths was 738. The fatality rate, that is, the number of

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deaths per hundred patients, was 16.5. The number of deaths and the fatality rate include all patients of all ages who died from the disease. The figure for the total number of cases includes all cases reported, whether or not the patients were treated.

All patients were treated with sulfonamide drugs. These chemicals need a minimal period of thirty-six hours to reach an adequate therapeutic level in the blood. Therefore, patients who died during the first day of treatment were not considered in the following statistical study, which attempted to evaluate the advantages of treatment with sulfonamide compounds.

At the Barros Luco Hospital patients below the age of 7 years are not admitted. In a series of 1,500 unselected patients 7 years of age and upward cared for in this hospital, there were 130 deaths—a fatality rate of 8.6 per cent. If we eliminate 37 patients who died within the first twenty-four hours, this percentage is reduced to 6.2. When these results are compared

TABLE 1.—*Age and Sex Distribution of 1,500 Patients Aged 7 Years or More*

Age, Yr.	Number of Patients	Proportion of Total Patients	Number of Deaths	Fatality Rate
7 to 10	147	9.7	17	10.8
10 to 20	553	37.1	46	8.3
20 to 30	433	28.8	15	3.4
30 to 40	208	13.6	11	5.5
40 to 50	102	6.6	23	22.5
Over 50	62	4.2	18	29.1

with the most recent foreign statistics, we can deduce that they are low, particularly because it is the largest experience published.

In infants under 4 years of age (treated and untreated) considered independently, the fatality rate was 28 per cent. In the patients treated at the Children's Hospital this rate was slightly lower. The Waterhouse-Friderichsen syndrome, a variety of the fulminant form, was observed with some frequency and was partially responsible for the increased fatality rate among infants. The distribution by age coincides with that generally described, with a proportional greater frequency of infants under 2 years of age.

The distribution by age of the series of 1,500 patients of 7 years and older which we have mentioned is shown in table 1.

One can clearly see that as age increases the number of reported cases decreases and the fatality rate rises. Meningococcic infection is, then, a grave disease in the extreme ages of life, while in adolescents and young adults, treated early, the prognosis is good.

Table 2 shows the age and sex distributions of the total 4,464 cases, as given in the official figures of the Board of Health of Chile (Province of Santiago). It is apparent that the first year of life contributed more cases than any succeeding one. In relation to sex we have recorded a slight predominance of men over women. No difference has been found in relation to fatality. It is interesting to recall that during the epidemic of Detroit from 1928 to 1931

TABLE 2.—*Age and Sex Distribution of 4,464 Cases*

Age Group	Estimated Population	Males		Females		Totals	
		Cases	Deaths	Cases	Deaths	Cases	Deaths
1941 *							
Under 1 yr.	37,120	15	9	6	4	21	13
1 to 4	123,938	33	6	18	4	51	10
5 to 9	121,705	21	3	32	4	53	7
10 to 14	111,473	29	5	17	2	46	7
15 to 19	142,372	22	7	15	2	37	9
20 to 24	143,747	10	2	15	6	25	8
25 to 34	228,258	20	3	8	1	28	4
35 to 44	156,112	11	1	6	2	17	3
45 to 54	103,688	5	2	4	2	9	4
55 to 64	55,730	1	1	1	1	2	2
Unknown	.....	1	..	..	..	1	..
Totals	1,261,717	168	39	122	28	290	67
1942							
Under 1 yr.	38,060	166	52	144	50	309	102
1 to 4	127,486	402	96	367	94	770	190
5 to 9	122,814	319	40	293	37	612	77
10 to 14	112,902	280	15	253	16	541	31
15 to 19	146,019	235	19	149	13	384	32
20 to 24	147,671	152	12	93	5	245	17
25 to 34	234,581	225	28	131	8	355	36
35 to 44	159,635	115	18	100	14	215	32
45 to 54	106,333	43	7	50	7	93	14
55 to 64	56,800	20	6	14	4	34	10
65+	38,306	7	3	15	9	22	12
Unknown	.....	5	..	1	..	6	..
Totals	1,290,607	1,978	296	1,610	257	3,586	553
1943 (to July 3)							
Under 1 yr.	38,765	38	21	26	6	64	27
1 to 4	129,827	65	15	40	12	105	27
5 to 9	125,096	51	12	43	7	94	19
10 to 14	114,978	43	9	32	2	75	11
15 to 19	148,748	32	5	30	1	62	6
20 to 24	154,456	28	3	13	2	41	5
25 to 34	238,758	51	5	25	3	76	8
35 to 44	162,413	30	3	11	2	41	5
45 to 54	108,144	18	7	3	..	21	7
55 to 64	57,818	1	2†	6	..	7	2
65+	39,021	1	1	1	..	2	1
Totals	1,318,024	358	83	230	35	588	118

* From Dec. 29, 1940 to Sept. 6, 1941 there were only 9 cases, with 2 deaths.

† In 1 case the diagnosis was made on January 2; this appears in the 1942 tabulation. The 1943 tabulation starts on January 9.

there was a proportion of men to women of 2 to 1. We have no opinion on the influence of the racial factor, because the population of Chile is all white.

From the standpoint of pathogenesis, we have adopted the designation "meningococcic infection" and not "meningococcic meningitis," because meningitis is always secondary and represents the localization of a more or less prolonged septicemia, as can be proved by a series of clinical and experimental facts. The old

term, "epidemic cerebrospinal meningitis," must be abandoned, for it does not correspond to an epidemiologic entity. In fact, in meningococcic infection the epidemic feature is not the number of patients but the number of carriers, for the number of carriers is much larger than the number of patients. It has been stated that an epidemic begins when the number of carriers exceeds 20 per cent of the population. In Chile, because of technical defects, this figure has been found to be only 10 per cent, but we feel sure that this is below the real proportion. If we consider a population of 1,290,000 people on the basis of 10 per cent, there must have been 129,000 carriers of meningococci, and we had only 4,464 patients. In other words, the sick people represent only about 0.3 per cent of the population, while the carriers constituted at least 10 per cent.

It is this character of meningococcic infection that causes the difficulties in epidemiologic control, because one can easily understand how impossible it would be to find and check all carriers. In this respect this disease behaves exactly like poliomyelitis.

Following this concept of the pathogenesis, we accept four clinical forms of evident disease, namely, (1) the acute, ordinary form, which occurs in the majority of cases of cerebrospinal meningitis and which is sometimes a mixed form of septicemia and meningitis (90 per cent of our cases were classified as of this form); (2) the septicemic form, acute or chronic. (We have encountered only a few cases of acute septicemia with positive blood cultures and in these the spinal fluid findings were negative; we have had no cases of the chronic type); (3) the fulminant form, which includes two types, (*a*) the Waterhouse-Friderichsen syndrome, in which the outstanding sign is hemorrhage of the skin and mucosa and particularly of the adrenals, and (*b*) the type in which the signs of unconsciousness, stupor, delirium and coma are predominant and patients fail in hours to death (almost all infants that died had one of these types); (4) the atypical forms, which do not fit the other forms described by one or another character of the clinical picture, such as total duration of the process, predominance of one symptom, mildness of the whole symptomatic complex, relapses or peculiar complications.

COMPLICATIONS

It is obvious that under the influence of chemotherapy the number of complications has diminished as compared with that in the era of serum therapy. We have classified the complications into two groups: (*a*) those related to the central nervous system and (*b*) those related to other organs.

In the first group we have seen: necrotic encephalitis, in those patients who had prolonged unconsciousness; central paraplegia (very rare); peripheral paralysis, and paresis of the cranial and spinal nerves, particularly those involving the intrinsic and extrinsic muscles of the eyes producing different known clinical pictures. The frequency of ocular complications was 5 per cent. Partial or total unilateral or bilateral deafness occurred in 5 per cent of cases. We were unable to determine whether deafness resulted from a lesion in the internal ear or from neuritis of the auditory nerve. However, in 1 patient meningococci were found at autopsy in a smear made from the fluid removed from the internal ear. Suppurative complications of the eye and ear were rare. We have recorded 1.2 per cent of cases with peripheral facial paralysis. Until now we have not found mental deficiencies as a complication of meningococcic infection. However, a psychiatrist is studying patients to determine mental aberrations on recovery and again at frequent intervals thereafter.

In the second group, arthritis was the commonest complication, with an incidence of 10 per cent. Arthritis involved one or more joints and varied from the simple painful joint to purulent arthritis. Pain was not severe compared with other arthritides. Arthritis occurred at any time in the disease, even while patients were receiving sulfonamide compounds or during convalescence. In an early stage of the disease it could be considered as metastatic and responded rapidly, in general, to sulfonamide drugs. In some of the cases we were able to isolate the meningococcus from the fluid removed. The pathogenesis of arthritic involvement late in convalescence is not clear, and we are not sure to what extent it is the consequence of sensitivity. Clinical progress was slow and was aided by heat, immobilization and removal of fluid, which at the same time relieved pain. Sulfonamide drugs have no influence on this type of arthritis. In general, both types recovered without sequelae. Other rare complications were: bronchopneumonia, myocarditis with arrhythmia, hepatitis with jaundice, urinary retention and decubitus ulcers.

DIAGNOSIS

We shall not consider the differential diagnosis but shall enumerate the laboratory procedures used in our hospital in the presence of a suspicious meningitis.

1. Spinal puncture was performed only for diagnostic purposes, to establish the cause of the

meningeal syndrome. If one considers that the causes of purulent meningitis other than meningococcic seem to increase during the epidemic period, this is the only way to reach an accurate diagnosis. During the epidemic, patients with the meningeal syndrome and a petechial rash were treated without lumbar puncture. We repeated the puncture only in patients who appeared not to respond to treatment.

The spinal fluid was examined chemically, microscopically and bacteriologically. From a practical point of view, during the epidemic meningitis with absence of organisms in the spinal fluid sediment and with negative cultures was considered as of meningococcic origin. Agents causing other forms of meningitis are usually demonstrable and sometimes exceed even the number of white cells in the fluid.

All the strains of meningococci isolated from our patients corresponded to group I of Griffith, which includes types I and III of Gordon and Murray. Group II strains were isolated from carriers. We did not isolate II alpha strains.

2. Cultures of blood and of bone marrow were also used. In an experimental study carried out on 81 patients the following results were obtained: positive blood cultures, 8 patients (9.7 per cent); positive bone marrow cultures, 12 patients (14.8 per cent). We have encountered this greater frequency of positiveness of bone marrow cultures in other infectious diseases, particularly in those produced by organisms of the typhoid-paratyphoid group. However, the number of positive blood cultures is too small, and we do not know whether this fact can be related to some technical failure or to an extreme susceptibility of the meningococci to the bactericidal power of the blood. It is worth noting that of the 81 patients 22 had evident clinical signs of sepsis and none of these had a positive blood culture considered isolatedly, but 5 had both cultures positive.

The nonprotein nitrogen levels of the blood and the spinal fluid were frequently increased. The determinations were of course performed on specimens obtained on the patients' admission, before treatment was started. With the Kahn test we had a relative frequency of false positive reactions of the general biologic type. Subsequently, the serologic control showed that these false positive reactions became negative. In other infectious diseases we have found 10 per cent of false positive reactions when the standard Kahn test was performed during the febrile period, and these always became negative during convalescence or slightly later. The verification test helped us in ruling out syphilis during the acute febrile period.

Each patient had also the following tests performed: complete urinalysis, determination of the blood sugar, Wassermann test, complete hematologic examination and sedimentation test.

PROGNOSIS

The prognosis depends on a number of factors —age, social condition, epidemic period. It has been demonstrated that symptoms are more severe during the first week of the epidemic and that they tend to become milder as it progresses; but the most important factors are undoubtedly the clinical form, the condition of the patient on admission and the number of days elapsed from the onset of the disease to treatment.

As a result of the good organization planned by the Board of Health under the direction of Dr. Eugenio Suarez, most of the patients were admitted within forty-eight hours from the onset.

When in cases of the common form chemotherapy was started a few hours after the onset, convalescence usually began by the fifth or sixth day.

With the common form the following symptoms and signs generally denoted a poor prognosis: (1) sudden change for the worse and progress to coma or collapse, showing acute adrenal failure; (2) psychosis, showing encephalitic involvement; (3) prolonged unconsciousness with high temperature in spite of treatment; (4) extreme tachycardia and low blood pressure; (5) heavy petechial rash or areas of ecchymosis.

With these prognostic criteria in mind and with the knowledge that the common form represents 90 per cent of cases, we are under the impression that treatment with sulfonamide compounds begun after an early and accurate diagnosis generally leads to a good prognosis for this disease at the present time.

TREATMENT

The treatment of meningococcic infection falls into three categories: (1) chemotherapy, (2) administration of antimeningococcus serum and (3) supportive treatment.

Undoubtedly, chemotherapy is today the most important element of treatment, as has been clinically and experimentally demonstrated. The general principles that determine the use of sulfonamide compounds are applicable to meningococcic infection, namely: (1) to obtain a sufficient concentration of active drug in the blood; (2) to maintain this concentration as long as

necessary, and (3) to control the amount and frequency of administration of the drug to avoid its toxic action.

The concentration of the drugs was obtained by giving intravenously 4 to 5 Gm. of the sodium salt to adults and 3 Gm. to adolescents. For children under 5 years the amount of drug given was usually 1½ to 2 grains per pound (0.10 to 0.15 Gm. per kilogram) of body weight. The concentration of the drug was maintained by repeating the dose every three or four hours. We have not employed the intrathecal route because we feel it is dangerous and without actual benefit. We know that all the drugs, possibly with the exception of sulfathiazole, diffuse readily to the spinal fluid. If patients were rational the oral route was preferred, the parenteral route being reserved for irrational patients or persons who could not retain fluids by mouth. Gavage was occasionally used. To adults with the grave forms of the disease we gave 12 Gm. a day orally, and to those with the ordinary form, 8 Gm. a day (1.5 or 1 Gm. every three hours respectively). Infants and children were given 0.06 to 0.13 Gm. (1 to 2 grains) per pound a day. These heavy doses were given until improvement occurred, and then the dose was gradually diminished, at the rate of 2 Gm. per day for adults, until all symptoms disappeared, according to the general principle that treatment with sulfonamide drugs should never be discontinued suddenly.

In our experience the average total dose for a patient was from 40 to 45 Gm. administered during eight to ten days. The extreme values were 20 Gm. and over 80 Gm. given from two to more than fifteen days.

For the control of the treatment and to avoid the toxic action of the drugs we have followed the criterion of clinical observation. The other criterion usually employed is the titration of the drug in the blood and in the spinal fluid. We did not do this routinely because with an average of 200 patients under treatment in our hospital and 100 in the Children's Hospital, there was no practical possibility of keeping such a routine control. Nevertheless, Prof. H. Alessandri, in a study on the variations of the levels of free and total drug in the blood and spinal fluid in a series of 200 patients, concluded:

> From a practical standpoint we must say that the clinical observation of the response to treatment is a more reliable index than a study of the levels obtained, for there is great individual variation. In fact, with the same daily doses there are great individual differences in levels found in the blood and the spinal fluid; but in the same patient there is a close relation between these figures. We believe, then, that a careful clinical observation is a good enough index to control the treatment, leaving titration only as a way to check whether the patient is receiving the drug.

We had the opportunity of using all the most common sulfonamide compounds except sodium sulfadiazine, which was not available in Chile when this work was done. With the idea of establishing which of the drugs was the most active in the treatment of meningococcic infection, a total of 450 patients was studied by division into three groups of 150 patients each, for treatment with sulfanilamide, sulfathiazole and sulfadiazine respectively. In this study we did not include sulfapyridine because our previous experience had shown its emetic properties which made strict control of the therapy difficult.

Our patients satisfied the same experimental conditions. They belonged to the same epidemic period, had more or less similar general conditions, were treated in the same hospital and were not selected, a fact of the utmost importance in a study of this kind. With this object the patients received a different drug in relation to their arrival to the hospital, regardless of their clinical condition. The first received sulfanilamide, the second sulfathiazole and the third sulfadiazine, the fourth again sulfanilamide, and so on.

With sulfanilamide the fatality rate was 13.3 per cent; with sulfathiazole, 10.7 per cent, and with sulfadiazine, 9.3 per cent. If we eliminate the patients who died within the first twenty-four hours, these fatality rates are reduced to 10.7 per cent for sulfanilamide, 6 per cent for sulfathiazole and 5 per cent for sulfadiazine. In relation to toxicity, 50 of the 150 patients treated with sulfanilamide showed toxic manifestations of one type or other. Sulfathiazole caused toxic reactions in 13 and sulfadiazine in only 7.

If we apply the chi square statistical criterion of expectancy, the differences in fatality rates are not significant. It has been clinically and experimentally demonstrated that sulfadiazine is far less toxic than sulfanilamide. This fact can also be deduced from our observations. Although there is no definite difference between the two drugs as far as bacteriostatic action is concerned, there is clear difference in relation to the toxic symptoms produced. The evidence favors the use of sulfadiazine as the drug of choice at the present time for the treatment of meningococcic infection. Sulfathiazole must be definitely included in the group of drugs that cure meningococcic infection, despite its low concentration in the spinal fluid.

With the exception of agranulocytosis we have seen all the complications described as due to sulfonamide drugs. With the exception of acute

hemolytic anemia in 1 instance, in none of our patients were these complications a serious problem.

In relation to serum, we are convinced that for treatment of the grave forms, particularly in aged persons, it is of value when given with the drug. With the use of rabbit serum (group I) we believe results will improve. In the cases of severe involvement we have also used blood transfusions and plasma as a vehicle for sulfonamide drugs with good results.

Supportive treatment consisted of administration of solutions of sodium chloride and dextrose, of vascular stimulants such as nikethamide and extract of adrenal cortex and of vitamin C, and good nursing care. For the irrational patients we have used phenobarbital and a combination of bromide and calcium galactogluconate intravenously and in rare occasions morphine.

SUMMARY

A major epidemic of meningococcic meningitis occurred at Santiago, Chile, during 1941 to 1943. The disease occurred in 4,464 persons, or 1 of every 300 inhabitants, was slightly more common in males than females and was more fatal in infancy and old age than in the other periods of life. The fatality rate for all patients was 16.5 per cent; for infants under 4 years, 28 per cent. In a series of 450 unselected patients treated with sulfonamide compounds the fatal rate was least (9.3 per cent) with sulfadiazine, greatest (13.3 per cent) with sulfanilamide and intermediate (10.7 per cent) with sulfathiazole. Toxic reactions were much more frequent (33.3 per cent) with sulfanilamide and least frequent (4.4 per cent) with sulfadiazine.

INCIDENCE OF FATTY LIVER IN TUBERCULOSIS WITH SPECIAL REFERENCE TO TUBERCULOUS ENTERITIS

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Fatty infiltration of the liver is a familiar feature of numerous diseases and has been recognized for many years as frequently seen post mortem in persons with tuberculosis. Curiously, the subject has received little attention, even though the development of hepatomegaly in tuberculous patients often presents diagnostic difficulties. At autopsy the demonstration of a huge fatty liver is always impressive and frequently not anticipated.

In the past such changes in the livers of tuberculous patients have been attributed to toxemia and anoxemia. Recently attention has been drawn to the development of fatty liver in a variety of conditions characterized by disordered metabolism. Numerous experimental studies have established this relationship and have served to heighten interest in its clinical application. The subject of fatty liver in tuberculous patients, accordingly, assumes new significance from the viewpoint of both therapy and pathogenesis.

The present study was stimulated by the observation of a group of patients in whom fatty liver was subsequently demonstrated at autopsy. Questions arose concerning the frequency of this type of hepatomegaly and its relation to the character of the pulmonary disease and extrapulmonary complications. As a preliminary step recent cases were reviewed. Several of these are reported to characterize the material under consideration.

CASE 1.—I. L., a 25 year old white woman, was admitted to the sanatorium in March 1938 with moderately advanced tuberculosis involving the upper third of the right lung. For about one year prior to admission she had complained of epigastric symptoms suggesting peptic ulcer. Anorexia and marked loss of weight had occurred. Shortly after her admission pneumothorax was induced on the right side and her prognosis was considered favorable. The sputum, which had contained tubercle bacilli on her admission, failed to reveal the organisms on direct examination or on culture. In spite of apparent control of the pulmonary lesion, her general condition failed to improve. Loss of weight continued, and weakness became a prominent symptom. In September 1938 diarrhea and colicky abdominal pain developed, which were attributed to intestinal tuberculosis. This impression was subsequently confirmed by roentgen studies of the gastrointestinal tract after administration of barium sulfate. At this time the liver was found to be enlarged, the edge being palpable at the iliac crest. It was painless, nontender, smooth and boggy. There was no jaundice. Shortly before death, on Oct. 10, 1938, the patient complained of sudden, sharp generalized abdominal pain and evidence of generalized peritonitis developed rapidly. Autopsy revealed effective collapse of the right lung. The intestine was ulcerated throughout its length, and at one point perforation had occurred. The liver was greatly enlarged, and microscopic study revealed massive deposition of fat within its cells.

CASE 2.—L. K., a 21 year old white woman, entered the sanatorium in June 1937, seven months after the onset of cough and malaise. Roentgen examination revealed far advanced tuberculosis with cavitation limited to the upper third of the left lung. Pneumothorax was unsuccessful, and temporary paralysis of the left side of the diaphragm was added to her regimen of strict rest in bed. Thoracoplasty was proposed but never performed because of her rapid downhill course. It is of interest that the pulmonary lesion remained essentially stationary. Abdominal symptoms were severe, and weakness and emaciation were noticeable. Terminally, there was pitting edema of the face and extremities. Death occurred on Sept. 4, 1938. At autopsy three small cavities were seen in the upper portion of the left lung, and scattered tubercles were demonstrated throughout the remainder of the lung. The right lung was essentially clear. Intestinal ulceration extended from a point 18 cm. below the duodenojejunal junction to the rectum, with almost complete denudation of the mucosa. The liver extended 10 cm. below the right costal border and was grossly and microscopically fatty.

CASE 3.—H. F., a 27 year old white man, entered the sanatorium in September 1937. By roentgen examination he was found to have moderately advanced tuberculosis confined mostly to the apex of the right lung. The sputum failed to reveal tubercle bacilli on smear or on culture. The right phrenic nerve was crushed in October. The course was uneventful until February 1938, when the patient began to complain of pain in the right lower quadrant of the abdomen, diarrhea and anorexia. Roentgen studies with the aid of barium sulfate revealed a small area of spasticity involving the terminal portion of the ileum and the cecum. About this time increase was noted in his pulmonary lesion and his sputum was found to contain tubercle bacilli. Attempts to establish pneumothorax

From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Mich.
*At the time the work reported here was done, Dr. Jones was associated with the Wm. H. Maybury Sanatorium, Northville, Mich.

were unsuccessful. From the onset of his abdominal symptoms until his death, five months later, the patient's clinical course was dramatic. He had a high, spiking fever with frequent shaking chills. Prostration and anorexia were extreme. During this interval the liver enlarged painlessly. Death occurred Aug. 16, 1938. Autopsy revealed extension of his disease bilaterally, with a small cavity in each lung. The pulmonary lesion, however, could still have been classified as moderately advanced and was entirely inconsistent with the hectic clinical course. Extensive ulceration involved the entire intestine below the duodenum. The liver was grossly and microscopically fatty.

In the management of these and of similar cases, it was felt that the character of the pulmonary disease was frequently inconsistent with the clinical course. The prominence of intestinal complication was impressive enough to suggest a possible relationship between this feature of the disease and the changes demonstrated in the liver.

Clinically, the enlargement of the liver was painless and unaccompanied by jaundice. The consistency was boggy and the edge of the liver smooth and rounded. In a number of cases it was possible to demonstrate the enlarged liver by fluoroscopic examination with the patient supine on the fluoroscope table. Anorexia and extreme weakness were prominent symptoms in the patients observed.

TABLE 1.—*Classification of 581 Autopsies According to the Extent of Enteritis and the Disposition of Fat in the Liver* *

Fat	Enteritis			Total
	None	Mild	Severe	
None	191 (157)	132 (141)	14 (39)	337
Mild	65 (76)	80 (68)	18 (19)	163
Severe	15 (38)	31 (34)	35 (9)	81
Total	271 (271)	243 (243)	67 (67)	581

* Expected numbers in parentheses.

From this preliminary survey the material was judged to be of sufficient interest to justify a statistical analysis of the autopsy records of the Wm. H. Maybury Sanatorium and the Herman Kiefer Hospital in Detroit. For this purpose reference was made to all autopsies performed on tuberculous patients at these two institutions up to January 1939. These included 642 consecutive autopsies, excepting 61 the records of which were found to be incomplete. From each case information was collected as follows: (1) type and extent of pulmonary tuberculosis as recorded by the pathologist; (2) presence and extent of intestinal tuberculosis; (3) gross and microscopic description of the liver. Microscopic sections were all inspected for the presence of fat or amyloid and roughly graded as to extent.

RESULTS

In the manner described observations were made on 581 cases. Fatty infiltration occurred in the liver in 244 cases (41.9 per cent). Amyloid was present in 37 cases (6.3 per cent). Intestinal tuberculosis occurred in 310 cases (53.3 per cent).

TABLE 2.—*Classification of 581 Autopsies According to the Extent of Enteritis and the Type of Pulmonary Disease*

Type of Pulmonary Disease	Enteritis			Total
	None	Mild	Severe	
Productive	81	35	4	120
Mixed	125	124	34	283
Exudative	65	84	29	178
Total	271	243	67	581

The association between the occurrence of enteritis and the presence of fat in the liver is set forth in table 1. The calculated values which one would expect if the relation were purely one of chance are enclosed in parentheses. Application of the chi-square test gives a value of 111.90, which shows that the variables are much more intimately associated than would be expected on the basis of chance. A value of 0.402 is obtained as a measure of association between the two variables, zero representing no association and 1 representing perfect association.

When the data of table 1 are further analyzed to measure the relationship of the extent of fatty change in the liver with enteritis, the type of pulmonary disease being constant, it was found that the presence of fat in the liver was definitely related to enteritis only when the associated pulmonary disease was "mixed" (chi-square value of 66.45) or exudative (chi-square value of 35.94). In the presence of productive disease this chi-square value dropped to 0.624, indicating a purely chance relationship.

TABLE 3.—*Classification of 581 Autopsies According to the Disposition of Fat in the Liver and the Type of Pulmonary Disease*

Type of Pulmonary Disease	Fat			Total
	None	Mild	Severe	
Productive	81	30	9	120
Mixed	169	74	40	283
Exudative	87	59	32	178
Total	357	163	81	581

The relationship between the type of pulmonary disease and the extent of enteritis is set forth in table 2. Some relationship appears to exist. Testing gives a chi-square value of 31.89 and a corresponding association value of 0.228.

The association between the type of pulmonary disease and fatty changes in the liver does not seem to be extensive, as set forth in table 3. Application of the chi-square test gives a value of 12.43. A value of 0.145 is obtained as a measure of association.

Microscopically, the fat was present in small droplets appearing first at the periportal areas and then extending to occupy the entire hepatic lobule in some cases.

The size of the liver when fatty changes were present was variable but difficult to evaluate precisely from the protocols. Frequently the liver was only moderately enlarged, but massive enlargement was occasionally noted. In a few instances the size was decreased.

In 16 of the 37 cases in which amyloid was present in the liver, this condition was associated with intestinal tuberculosis. Of the entire group of 310 cases in which enteritis occurred, amyloid was present in 5 per cent, while fatty changes occurred in 164 cases, or 53 per cent. The likelihood is thus suggested that hepatomegaly occurring in patients with tuberculous enteritis is more apt to be due to fat than to amyloid.

COMMENT

The incidence of fatty infiltration of the liver in tuberculous patients coming to autopsy was found to be 41.96 per cent. This is consistent with results of earlier observers. Ullom [1] cited 35 patients with such infiltration in a series of 100 patients dying with tuberculosis, and Rolleston and McNee, quoting Louis,[2] mentioned 40 in a series of 120. Recently Parini [3] examined the livers of 50 patients with chronic ulcerative intestinal tuberculosis and of a control series of 50 persons with active tuberculosis without intestinal disease. He concluded that in cases of ulcerative intestinal tuberculosis there are always fairly severe fatty changes in the hepatic parenchyma. Characteristically he found these changes involving the peripheral portion of the hepatic lobule. He expressed the belief that there is a certain parallelism between the gravity of the intestinal lesion and the degenerative changes in the liver.

In the past there has been considerable speculation regarding the pathogenesis of this lesion, with most authors attributing it to anoxemia or to general toxemia. The former theory may be dismissed briefly by calling attention to the infrequent occurrence of fatty liver in such diseases as pulmonary emphysema, in which there is long-standing anoxia. The case for general toxemia is stronger since fatty liver frequently occurs in association with acute infectious diseases.

From the point of view of the present data greater significance should probably be attached to the established occurrence of fatty liver in persons who have died of chronic ulcerative colitis.[4] Here there exists an obvious parallel with tuberculous enteritis. Food intake is limited in amount and character over a prolonged period of time, and diarrhea interferes with normal absorption of food from the intestine. Possibly toxins are absorbed from the intestinal tract by way of the portal circulation. Since the earliest deposition of fat within the liver occurs in the periportal areas, this possibility should be borne in mind.

It should be pointed out that the classification of pulmonary disease as "mixed," while useful for statistical study, is somewhat misleading. By definition the so-called "mixed" disease is that in which both productive and exudative lesions were present. Patients with such involvement might be expected to exhibit the clinical and pathologic features of the exudative, or more active, lesions. In other words, extrapulmonary changes secondary to exudative disease should occur in both the "mixed" and the exudative type. For purposes of discussion, then, these two types may be considered together.

Analysis of various statistical relations has demonstrated several facts. It was shown that fatty liver is found frequently, though not always, in patients with tuberculous enteritis, this association being of significance only when the accompanying pulmonary disease is exudative or "mixed." The conclusion from this observation is that the hepatic and intestinal lesions are concomitant pathologic processes secondary to the pulmonary lesion.

Actually, there were a number of striking exceptions to this generalization. Occasional patients were found showing massive fatty infiltration of the liver with normal intestinal mucosa or with accompanying pulmonary lesions clinically controlled. On the other hand, the liver was frequently found to be normal even in the presence of extensive enteritis and fulminating pulmonary disease. Obviously, any explanation must be sufficiently broad to include these exceptions.

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2. Rolleston, H. D., and McNee, J. W.: Diseases of the Liver, Gallbladder and Bile Ducts, ed. 3, London, The Macmillan Company, 1929.

3. Parini, F.: Studio anatomo-istologico del fegato nella enterite tubercular cronica, Sperimentale, Arch. di biol. **95**:65-98, 1941.

4. Kaufman, E.: Lehrbuch der speziellen pathologischen Anatomie, Berlin, W. de Gruyter & Co., 1922.

Further examination of the material, particularly of the patients with conditions representing the exceptions to the general rule, disclosed the apparently constant presence of extreme emaciation in patients with fatty liver. The degree of emaciation was difficult to estimate from the autopsy protocols, but its unusual extent in these patients was noted with great frequency. This suggests an underlying metabolic factor. Such a concept adds clinical significance to the statistical results. In patients with severe enteritis, absorption of food material may be disturbed by rapid passage of food through the intestine and by damage to the intestinal wall. Moreover, these patients suffer extreme anorexia and the ingested food is consequently restricted in quantity and quality. Vomiting is frequently important. If the concomitant pulmonary disease is exudative or "mixed," the toxemia becomes greater, causing still further disturbance in the appetite.

All this is postulated in the light of recent evidence indicating the metabolic basis of fatty liver in depancreatized animals,[5] in persons with chronic alcoholism,[6] and with pellagra,[7] in diabetic children[8] and in experimental animals maintained on diets deficient in vitamin C,[9] high in cholesterol, low in protein or containing "lipotropic" protein.[10] It is obvious that additional information must be obtained from careful clinical observations to support the implications in this study. From a practical point of view, however, it seems justifiable to attempt to formulate some clinical concept of fatty liver as it develops in persons with tuberculosis. This material suggests that such changes may be expected in patients in whom inanition persists for a long time.[11] Intestinal tuberculosis as a cause of severe inanition plays an important contributory role.

Reports are now available to demonstrate the reversibility of fatty infiltration of the liver in various other diseases. The implication would seem to be that the high incidence of fatty liver in tuberculous patients is a significant commentary on the need for greater attention to the maintenance of adequate nutrition during the course of the disease, particularly for the patients with tuberculous enteritis. For this group the application of dietary principles used in the treatment of typhoid, chronic ulcerative colitis and similar diseases may prove efficacious in the prevention of fatty liver as a feature of malnutrition.

SUMMARY

The cases described illustrate the occurrence of fatty infiltration of the liver in persons with tuberculosis. Material was studied from 581 autopsies on tuberculous patients. Fat was noted in the liver in 41.9 per cent of them.

Extensive fatty infiltration of the liver was often associated with extensive tuberculous enteritis.

Fatty liver and tuberculous enteritis were more apt to occur jointly when "mixed" or exudative types of pulmonary disease existed.

Extreme emaciation appeared to be a constant accompaniment of fatty liver.

An effort was made to evaluate this material in the light of current concepts of fatty liver.

Dr. G. E. Harmon assisted in arranging and interpreting the statistical material.

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7. Sydenstricker, V. P.; Schmidt, H. L., Jr.; Geeslin, L. E., and Weaver, J. W.: Liver in Pellagra, Am. J. M. Sc. **197**:755-763 (June) 1939.

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9. Beyer, K. H.: Protective Action of Vitamin C Against Experimental Hepatic Damage, Arch. Int. Med. **71**:315-324 (March) 1943.

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RECENT ADVANCES IN PHYSIOLOGY OF THE THYROID AND THEIR CLINICAL APPLICATION

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CHICAGO

In times past there was much discussion of such questions as the differences between exophthalmic goiter and toxic nodular goiter. More recently it has been generally agreed that the differences in the history and the pathologic picture of these variations of hyperthyroidism are probably due to such factors as the age of the patient, the duration of the disease, the number of times acute exacerbations have occurred and the history with respect to intake of iodine, etc. From the functional point of view, most students of such disease have been content to differentiate between hyperthyroidism and hypothyroidism, and have bent their efforts toward elucidation of the causation and the physiologic manifestations of these abnormal functional states.

A salient fact in physiology of the thyroid has been the influence of iodine on the function of the gland. This relationship has not been easy to interpret. It seemed fairly certain that the colloid material within the thyroid follicles represented the thyroid hormone or a precursor of it. But it was difficult to explain why, on the one hand, a *lack* of iodine led to an accumulation of the colloid (colloid goiter), while, on the other hand, the *therapeutic use* of iodine in hyperthyroid states in which there was a paucity of colloid led to a reaccumulation of this material.

Figure 1 is a reproduction of an illustration from Means's text on "Thyroid and its Diseases," showing "The Several Phases of the Thyroid Follicle." Section *a* represents the fetal type of follicle, which contains no colloid. Section *b* shows the normal adult follicle lined by cuboidal epithelium. It is filled with colloid which is moderately vacuolated. The colloid of the normal follicle is characterized chemically by a relatively high iodine content. Section *c* represents the so-called hyperplastic follicle. It is the characteristic picture found in states of hyperfunction, although it may also occur at a time when no excess of thyroid hormone is being liberated by the gland. The cells lining the hyperplastic follicle are columnar and are increased in number. The increase in the perimeter of the follicle together with the depletion of its colloid content leads to infolding of the wall of the follicle. Section *d* shows the hyperplastic follicle after administration of iodine. The essential change is the increased amount of colloid, which fills the follicle and straightens out its walls. Note the high vacuolation of its colloid. Section *e* represents the end result of long-continued lack of iodine. Here the follicle is filled with colloid to a degree which stretches its epithelium flat. There are no vacuoles, and the colloid is chemically characterized by a low iodine content.

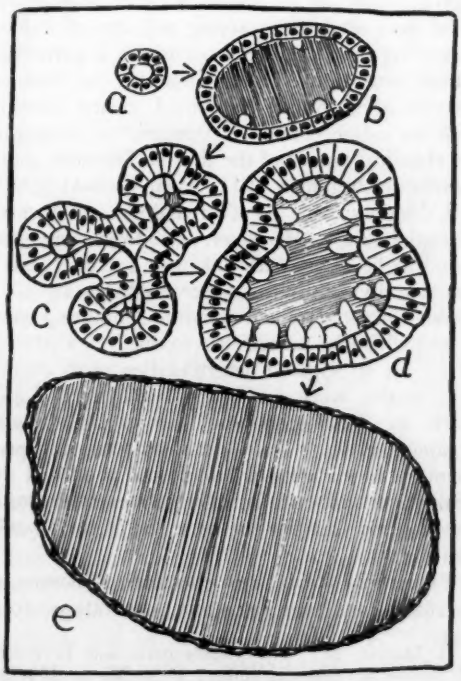

Fig. 1.—Drawings showing "the several phases of the thyroid follicle" (from Means [2]). See the text for explanation of the parts.

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Based on the Lippitt Memorial Lecture of the Mount Sinai Hospital, Milwaukee, delivered by Dr. Soskin at the Marquette Medical School Auditorium, Jan. 18, 1944.

There have been a number of attempts to rationalize these various histologic pictures. A notable effort was Marine's [1] description of the thyroid cycle, which undoubtedly presented the correct sequence of events but offered no explanation. Means [2] attempted to explain the morphologic phenomena on the basis of the need of the body for thyroid hormone and the efforts of the thyroid gland to compensate for the lack of iodine by the production of excessive amounts of iodine-poor colloid. Recent physiologic knowledge may make it possible to supersede this teleologic explanation with a more factual one.

In 1899, Oswald [3] demonstrated that the colloid material contained in the thyroid follicle was composed largely of a protein resembling the globulins in its physical characteristics. This thyroglobulin, as he named it, was later found to be associated with varying amounts of iodine, depending on the gland from which a particular sample was derived.[4] In general, the biologic activity of a sample of colloid varied directly with its iodine content. Attempts to determine the chemical nature of the thyroid hormone more specifically began in 1911, when Oswald identified diiodotyrosine as a breakdown product of thyroglobulin.[5] However, diiodotyrosine was found to be biologically inactive, and the search for the thyroid hormone continued. In 1915 Kendall isolated an active iodine-containing compound which he named "thyroxine." [6] Unfortunately his attempts at determination of its chemical identity were not quite successful. Later work by Harington and Barger [7] met with greater success. They established the accepted chemical formula for thyroxin and produced the compound synthetically. Until recently thyroxin was considered to be the hormone of the thyroid gland.

Figure 2 shows the chemical structure of thyroxin and indicates its derivation from tyrosine and inorganic iodine, through a diiodotyrosine stage. It should be realized that these transformations occur within the molecule of thyroglobin as inorganic iodine reacts with it. The dissolution of the large thyroglobulin molecule is necessary for the laboratory separation and identification of its constituents, but it is not necessary for the formation of its biologically active components.

On the basis of these facts one might expect that the biologic activity of a given dose of desiccated thyroid would depend on its thyroxin content. As a matter of fact, however, Means and his co-workers [8] demonstrated that the calorigenic action of desiccated thyroid could not be accounted for by the thyroxin it contained. It ran more parallel with its total organic iodine content. This seemed to indicate that thyroglobulin rather than thyroxin was the actual thyroid hormone. But this supposition was shattered by Lerman,[9] who tried to demonstrate the presence of thyroglobulin in the blood of both normal and hyperthyroid persons, using immunologic methods of the highest sensitivity. He was unable to demonstrate the presence of any thyroglobulin in the blood. This raised two possibilities: first, that the thyroid gland contained another active material in addition to thyroxin, and, second, that the activity of administered thyroglobulin depended on the preformed

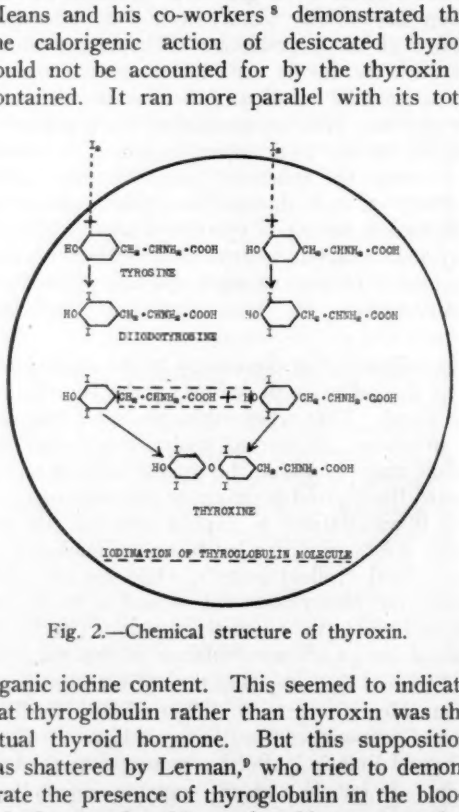

Fig. 2.—Chemical structure of thyroxin.

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2. Means, J. H.: The Thyroid and Its Diseases, Philadelphia, J. B. Lippincott Company, 1937.

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thyroxin which it contained plus the thyroxin formed from its residual organic iodine. In view of the fact that no active constituent of thyroglobulin other than thyroxin has yet been demonstrated, the second explanation appears to be the correct one.

Although the thyroid hormone does not enter the blood stream as thyroglobulin, neither does it circulate in the blood as free thyroxin. This was made clear by considerable work on the nature of the iodine fractions in the blood in health and in disease, a subject of some practical interest to the clinician. Salter has summarized this work in his recent monograph "The Endocrine Function of Iodine."[10] Briefly, iodine exists in the blood in both inorganic and organic forms. The total iodine content of the blood bears some relationship to the functional activity of the thyroid gland when the exogenous intake of iodine is consistently low. The total blood iodine varies from 5 to 15 micrograms per hundred cubic centimeters in the normal person. In the hyperthyroid state it ranges from 15 to as high as 110 micrograms per hundred cubic centimeters. In hypothyroidism the values vary from 2 to 5 micrograms. However, wide fluctuations in all these values may be caused by inadvertent intake of iodine-containing or iodine-enriched foods. The organic, or combined, form of iodine in the blood may be separated as two fractions, the so-called D form (diiodotyrosine) and the T form (thyroxin). These organic fractions are not present in the blood as free diiodotyrosine and thyroxin respectively but exist in combination with other proteins and are precipitated together with the proteins of the blood. The T fraction of the combined iodine is much less subject to unexplained fluctuations and behaves in a manner consistent with the behavior of thyroid hormone. In normal persons the T iodine is about one third of the total iodine and ranges from 2.9 to 4.8 micrograms per hundred cubic centimeters. It rises in hyperthyroidism and falls when iodine is administered to hyperthyroid persons. In myxedema the T iodine fraction may approach zero. In view of these facts it may be seen that determination of the blood iodine, and particularly of the T iodine fraction, can be a useful clinical procedure in diagnosis and therapy. Unfortunately, however, the chemical methods are not yet practical for routine clinical use. It is to be hoped that these methods will be further simplified in the future.

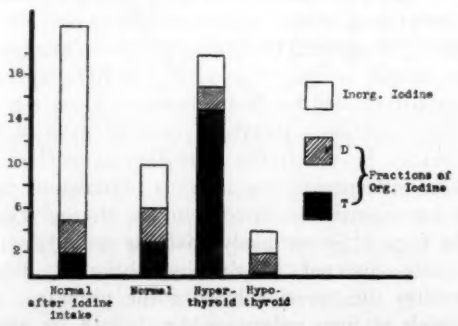

Fig. 3.—Iodine fractions of the blood under various conditions.

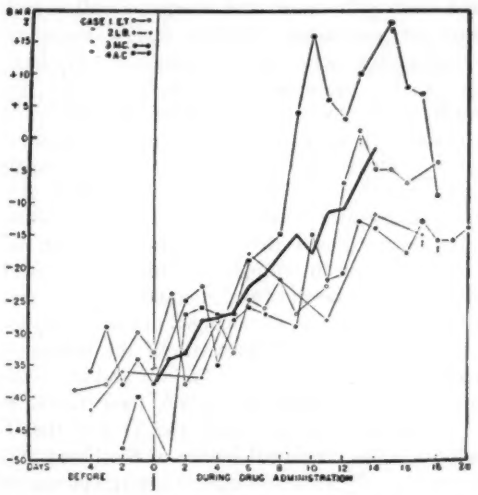

Fig. 4.—Metabolic response of 3 patients with myxedema and 1 with cretinism receiving an alkaline (baryta) hydrolysate of iodinated serum protein. The heavy solid line is a standard curve of reference representing the metabolic response to the daily administration of thyroxin polypeptide containing 0.5 mg. of iodine. (From Salter, W. T., and Lerman, J.: Tr. A. Am. Physicians **53**:204, 1938.)

One of the most startling recent developments had been the finding that the thyroid hormone can be made by tissues other than the thyroid gland. Indeed it is amazingly easy to produce by incubating blood proteins and inorganic iodine in a test tube. What is more, casein can be substituted for the blood proteins and thyroxin will still appear. This phase of knowledge of the thyroid started with the work of Abelin and others,[11] who showed that iodinated egg albumin or serum proteins caused a rise in the

10. Salter, W. T.: The Endocrine Function of Iodine, Cambridge, Mass., Harvard University Press, 1940.

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basal metabolic rate when fed to experimental animals or to myxedematous human beings (fig. 4). Several years later, Ludwig and Mutzenbacher,[12] investigated a crystalline precipitate which formed in an iodinated casein solution and found it chemically identical with thyroxin. Reinecke and his associates [13] have since investigated the conditions influencing the yield of thyroxin in vitro. They found they could start with casein and inorganic iodine and obtain an iodocasein which is several times as physiologically active as thyroglobulin. Hydrolysis of this material yields eighty times the amount of thyroxin which may be obtained from an equal weight of desiccated thyroid gland.

It is thus clear that thyroxin can be made outside the thyroid gland. Is it so made in the living organism? There can be no doubt of it. It has been shown that the administration of inorganic iodine to completely thyroidectomized animals will raise the basal metabolic rate [14] and will produce the specific changes in the epiphyses of the long bones characteristic of the action of thyroxin.[15] Furthermore, Chaikoff and his associates,[16] using radioactive iodine, have found it to be incorporated into both the D and the T fraction of the combined iodine of the blood and tissues in thyroidectomized-hypophysectomized animals. What, then, is the function of the thyroid gland? Is it merely a collection depot for the hormone which is formed elsewhere? Probably not. Administered inorganic iodine accumulates in the normal thyroid gland at a rate eighty times that at which might be expected to enter the gland on the basis of a uniform distribution throughout the body. The hyperplastic thyroid gland takes up iodine three hundred to four hundred times as rapidly as do other tissues.[17] It appears, therefore, that the thyroid is a specialized organ which can manufacture thyroxin much more rapidly than any other tissue, even though it is not exclusive in its ability to make the hormone. This suggests interesting possibilities as regards the other endocrine glands. If it is generally true, there need no longer be any difficulty in explaining how the simpler forms of life can get along nicely without the help of endocrine glands; or, as Means puts it, "The hormone precedes the gland in the evolutionary scale." [18]

This newer knowledge of physiology of the thyroid has not shed too much light on the cause of clinical disturbances of the thyroid. The depression of activity of the thyroid accompanying destructive disease of the pituitary gland has long suggested the possibility that hyperfunction of the thyroid might be due to a stimulation originating in the hypophysis. More recently, thyrotropic extracts of the anterior lobe of the pituitary have been prepared, and it has been possible by suitable assay methods to demonstrate that the amount of thyrotropic hormone in the circulating blood varies with the functional state of activity of the thyroid gland. On the other hand, a frequent clinical observation in regard to the cause of hyperthyroidism is the presence of mental and emotional strain in the preceding history. Experimental work has shown that it is possible for excessive nervous stimulation to result in permanent functional hyperactivity of the gland. Thus Friedgood and Cannon [19] have shown that in an occasional animal continued stimulation of the cervical sympathetic nerve (following its anastomosis with the right phrenic nerve) may result in a syndrome which duplicates the essential features of exophthalmic goiter in human beings. Conversely, bilateral cervical sympathectomy has been shown to lower significantly the basal metabolic rates of most of the animals in which the operation is performed. The influence of the nervous stimulation need not necessarily be directly on the thyroid gland. In fact, it seems likely that the stimulation is finally directed to the hypothalamus, which excites the anterior lobe of the pituitary, and which in turn influences the thyroid by means of the trophic hormone we have already discussed.

Although the causation of clinical disturbances of the thyroid can be discussed only in terms of possibilities at the present time, a great deal has been learned concerning the regulation of thyroid

12. Ludwig, W., and Mutzenbacher, P.: Herstellung von Thyroxin, Monojodtyrosin und Dijodotyrosin aus jodiertem Eiweiss, Ztschr. f. physiol. Chem. **258**:195, 1939.

13. Reineke, E. P., and Turner, C. W.: The Recovery of Crystalline Thyroxine from Iodinated Casein, J. Biol. Chem. **149**:555, 1943.

14. Chapman, A.: Extrathyroidal Metabolism of Iodine, Surg., Gynec. & Obst. **74**:483, 1942.

15. Silberberg, M., and Silberberg, R.: Effect of Potassium Iodide on Bone and Cartilage in Thyroidectomized Immature Guinea Pigs, Arch. Path. **28**:846 (Dec.) 1939.

16. Morton, M. E.; Chaikoff, I. L.; Reinhardt, W. O., and Anderson, E.: Formation of Thyroxine and Diiodotyrosine by Completely Thyroidectomized Animals, J. Biol. Chem. **147**:757, 1943.

17. Hertz, S.; Robert, A., and Salter, W. T.: Radioactive Iodine as an Indicator in Thyroid Physiology: IV. The Metabolism of Iodine in Graves' Disease, J. Clin. Investigation **21**:25, 1942.

18. Means, J. H.: Some New Approaches to the Physiology of the Thyroid, Ann. Int. Med. **19**:567, 1943.

19. Friedgood, H. B., and Cannon, W. B.: Autonomic Control of Thyroid Secretion, Endocrinology **26**:142, 1940.

activity. This information gives a greater insight into what goes on once thyroid disease is established, and will no doubt soon lead to a final solution as regards causation. For example, it was not long after the discovery of the thyrotropic hormone that attempts were made to determine its level in the blood and the urine in normal and in pathologic thyroid states. Surprisingly, it was found that there was a decreased amount of thyrotropic hormone in hyperthyroidism and an increased amount in hypothyroidism.[20] This seemed to contradict the idea that the thyroid gland was being overstimulated or understimulated by the pituitary. Indeed, far from being responsible for the clinical syndromes, the pituitary seemed to be making every effort to compensate for them. More recently, however, Rawson and his group [21] demonstrated that thyrotropic hormone is inactivated by incubation with slices of thyroid tissue in vitro. They further reported that hyperplastic and hyperfunctioning thyroid tissue has a greater inhibitory action on thyrotropic hormone, while hypofunctioning thyroid has practically no inhibitory effect (fig. 5). The inactivation depends on an oxidation, probably of an SH to an S-S configuration, and the inactivated thyrotropic hormone can be reactivated by suitable chemical measures.[18] This work indicates the necessity for a reinvestigation of hypophysial-thyroid regulation, using the total thyrotropic content of blood and urine (active plus reactivated) as an index of the regulatory activity of the pituitary.

Assuming that further investigation will confirm the probability that the functional activity of the thyroid gland will be found to vary directly with the total amount of the thyrotropic hormone, there is a counterregulatory phenomenon to be considered, namely, the influence of the thyroid hormone on the pituitary gland. This has been shown in two ways: first, the pituitary glands of thyroid-treated animals have

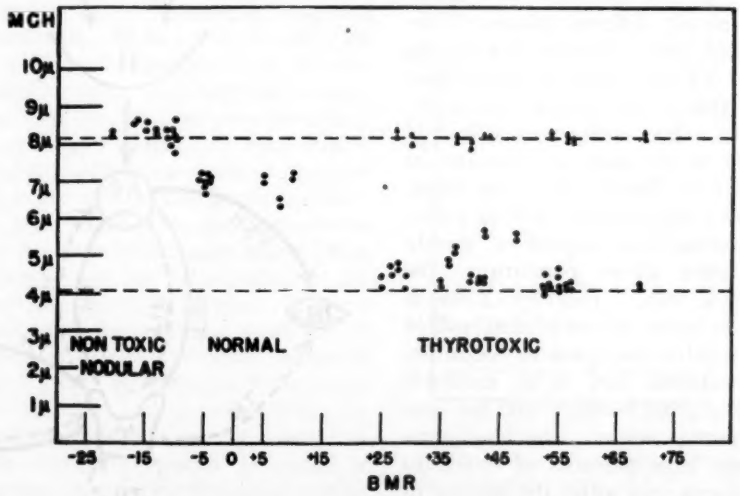


Fig. 5.—Response of chick thyroid to injection of mediums containing thyroid-stimulating hormone after these had been exposed to human thyroid tissue explants. (From Rawson and others.[21])

a lower content of thyrotropic hormone than do normal glands [22]; secondly, thyroid hormone may actually influence the pituitary to produce a thyroid-depressing substance. Reforzo-Membrives [23] has shown that the administration of preparations of pituitary glands taken from animals previously treated with thyroid extract resulted in a depression of thyroid activity in the recipient animals.

Another regulatory action is probably exerted by the level of thyroid hormone in the blood on

20. Hertz, S., and Oastler, E. G.: Assay of Blood and Urine for Thyrotropic Hormone in Thyrotoxicosis and Myxedema, Endocrinology **20**:520, 1936. Rawson, R. W., and Starr, P.: Direct Measurement of Height of Thyroid Epithelium: A Method of Assay of Thyrotropic Substance; Clinical Application, Arch. Int. Med. **61**:726 (May) 1938.

21. Rawson, R. W.; Graham, R. M., and Riddell, C. B.: The Effect of Normal and Pathological Human Thyroid Tissues on the Activity of the Thyroid Stimulating Hormone, Ann. Int. Med. **19**:405, 1943.

22. Kuschinsky, G.: Ueber die Bedingungen der Sekretion des thyreotropen Hormons der Hypophyse, Arch. f. exper. Path. u. Pharmakol. **170**:510, 1933.

23. Reforzo-Membrives, J.: Thyroid-Inhibiting Action of Hypophyses of Rats Fed with Thyroid, Endocrinology **32**:263, 1943.

the thyroid gland itself. Thus Loeb and others [24] have shown that the simultaneous administration of thyroid hormone with thyrotropic hormone may prevent the stimulation of the thyroid gland which would occur if the thyrotropic hormone were given alone. Similar effects have been demonstrated in vitro. Galli-Mainini [25] has reported that while the addition of thyrotropic hormone to slices of thyroid gland increases the oxygen (O_2) consumption of the tissue, the addition of thyroglobulin results in a decreased oxygen consumption.

One of the paradoxes in the regulation of thyroid activity has been that connected with the use of iodine. On the one hand, adequate iodine intake prevents or reduces colloid goiters; on the other hand, iodine is also used for temporary control of the hyperplastic and hyperfunctioning gland. Marine's dictum that these two pathologic states are merely different phases of the same type of disturbance helped to resolve the contradiction but did not explain the mechanism. It now appears that in the presence of an increased supply of iodine, iodine-poor colloid is further iodinated to the stage of thyroxin, in which form it can be liberated from the gland. Since the previous difficulty was lack of iodine and not a disturbance in regulation, merely supplying the iodine allows a return to the normal physiologic state. However, there is some evidence in cases of established colloid goiter that too rapid or too great an iodination may in some instances lead to an excessive formation of the thyroid hormone and the precipitation of hyperthyroidism. The administration of relatively large amounts of iodine to hyperthyroid persons may affect the amount of thyroxin liberated from the gland in an entirely different way. Reineke and co-workers [26] have shown that the progressive iodination of casein produced compounds with progressively greater thyroid activity until the iodine content reached 7 per cent. As the proportion of iodine was increased beyond this figure the thyroid activity of the end product was reduced. The same reaction to relatively large amounts of iodine may occur within the living gland.

Figure 6 modified from Means,[18] summarizes diagrammatically the various known mechanisms included in the complicated regulating system which controls the activity of the thyroid gland.

We are now ready to discuss the medical treatment of hyperthyroidism. In this regard, one of the important facts discovered within recent years is that many of the classic signs and symptoms of the disease are not direct consequences of the excessive amounts of circulating thyroid hormone. For example, exophthalmos, when it occurs, is due to the influence of the thyrotropic hormone of the anterior lobe of the pituitary.[27]

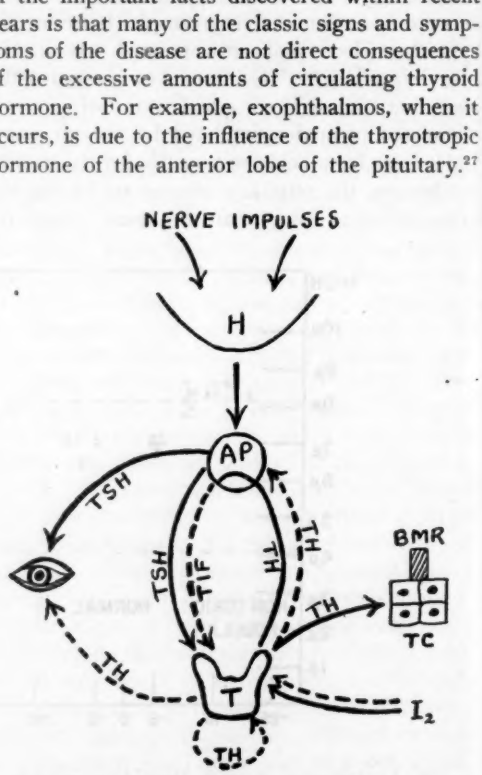


Fig. 6.—Regulation of the activity of the thyroid (modified from Means [18]). *H* indicates hypothalamus; *AP*, anterior lobe of the pituitary; *T*, thyroid; *TC*, tissue cells; *TSH*, thyroid-stimulating hormone; *TH*, thyroid hormone; *TIF*, thyroid-inhibiting factor; I_2, inorganic iodine.

As a matter of fact, there is some evidence that thyroxin actually counteracts the influence of the thyrotropic hormone on the eyes. Thus, large doses of thyroxin administered to normal animals do not usually lead to exophthalmos, while, on the other hand, thyrotropic hormone will cause exophthalmos in thyroidectomized animals. In these same animals the administration of thyroxin together with thyrotropic hormone will prevent

24. Loeb, L.; Bassett, R. B., and Friedman, H.: Further Investigations Concerning the Stimulating Effect of Anterior Pituitary Gland Preparation on the Thyroid Gland, Proc. Soc. Exper. Biol. & Med. **28**:209, 1930. Aron, M.: Particularités histologiques de la reaction de la thyroïde aux extraits de lobe anterieur d'hypophyse, Compt. rend. Soc. de biol. **103**:145, 1930.

25. Galli-Mainini, C.: Effect of Thyroid and Thyrotropic Hormones upon Oxygen Consumption (Q_{O_2}) of the Thyroid of the Guinea Pig, Endocrinology **29**:674, 1941.

26. Reineke, E. P., and Turner, C. W.: Effect of Progressive Iodination on Thyroidal Activity of Iodinated Casein, J. Biol. Chem. **143**:285, 1942.

27. Marine, D., and Rosen, S. H.: Exophthalmos in Thyroidectomized Guinea Pigs by Thyrotropic Substance of Anterior Pituitary, and the Mechanism Involved, Proc. Soc. Exper. Biol. & Med. **30**:901, 1933.

the exophthalmos that would otherwise occur.[28] There is of course little that can be done about persistent exophthalmos in a medical way until a safe method for depressing the hypophysis or neutralizing the thyrotropic hormone is found.

It is now known that a number of other features of the classic syndrome of hyperthyroidism are actually secondary effects of the disturbance due to vitamin deficiencies. The increased basal metabolic rate of hyperthyroidism leads to a greater requirement for practically all the vitamins. Unless the excessive caloric expenditure is accompanied by a corresponding intake of food, and particularly of the accessory food factors, avitaminoses enter the picture. The key vitamins are therefore those of the B complex, for it is relative lack of these which leads to decreased appetite. The poor appetite leads to a decreased intake of food and of accessory factors; the appetite is even further reduced, and a vicious cycle is established. It has been shown that administration of ample amounts of the B complex to experimentally hyperthyroid animals prevents loss of weight and may even allow continued gain in weight despite the hypermetabolism.[29] Depletion of hepatic stores of glycogen is prevented, as are also the characteristic disturbances in the sex cycle.[29] It is also claimed that there is some decrease in the pulse rate. These effects are largely but not wholly due to the thiamine component of the B complex. It seems clear, however, that the muscular weakness of hyperthyroidism is due to a relative deficiency of pyridoxine (vitamin B_6). Figure 7 illustrates the effect of treatment with pyridoxine on the ability to perform work in a case of ours.[30] It may be seen that this vitamin permitted a dramatic increase in work performance during a period when compound solution of iodine was not given and when there was no significant change in the basal metabolic rate. In the subsequent period, after treatment with strong solution of iodine U. S. P. was begun, cessation of pyridoxine treatment led to a decreased ability to perform work, even though the basal metabolic rate was falling.

When intake of vitamin B is not supplemented in a case of hyperthyroidism and food intake is relatively diminished, other vitamin deficiencies appear. The deficiency of vitamin C accounts in part for the creatinuria which is characteristic of the disease. It has been shown that the administration of adequate amounts of vitamin C causes a significant reduction in excretion of creatine.[31] The negative calcium balance of hyperthyroidism is probably wholly due to a relative deficiency of vitamin D, for the calcium balance can be restored to normal by treatment with this vitamin.[32]

The accompanying table lists the signs and symptoms of hyperthyroidism and classifies them according to whether they are direct, partly indirect or completely indirect effects of the excessive thyroid hormone activity. It is clear that at the present time treatment of hyperthyroidism should include prompt correction of the relative vitamin deficiencies.

The information thus far outlined has led to a better understanding of thyroid disease and to

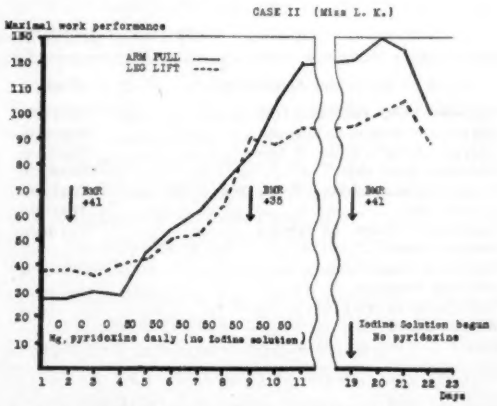


Fig. 7.—Effect of treatment with pyridoxine on the ability to perform work.

improved medical management as far as preoperative and postoperative care is concerned. It has not obviated the necessity for surgical treatment in the majority of cases. However, another series of studies gives promise of a real medical treatment for hyperthyroidism and indeed is under way clinically at the present time.

This work began somewhat fortuitously with the observation by Kennedy that rabbits fed on rape seed acquired definite goiters. He subsequently determined that it was the thiourea content of the seed which was responsible for the effect, and he was able to produce goiters

28. Smelser, G. K.: Study of Retrobulbar Tissues in Experimental Exophthalmos in Guinea Pigs, Am. J. Anat. **72**:149, 1943.

29. Drill, V. A.: Interrelations Between Thyroid Function and Vitamin Metabolism, Physiol. Rev. **23**: 355, 1943.

30. Rosenbaum, E.; Portis, S., and Soskin, S.: The Relief of Muscular Weakness by Pyridoxine Hydrochloride, J. Lab. & Clin. Med. **27**:763, 1941.

31. Fischer, G., and Oehme, C.: Vitamin C and thyreotoxische Kreatinurie, Klin. Wchnschr. **16**:1453, 1937.

32. Pugsley, L. I., and Anderson, E.: Effect of the Administration of Calciferol on Increased Calcium Excretion Induced by Thyroxine, Biochem. J. **28**:1313, 1934.

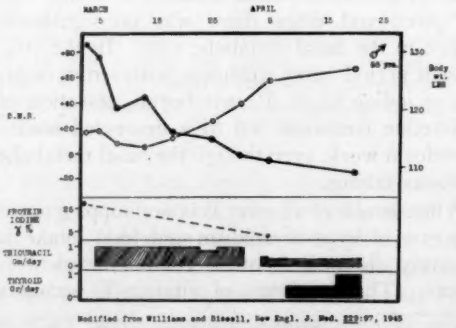
by feeding allyl thiourea.[33] Richter [34] obtained identical results by using phenylthiourea in rats. Both authors reported that histologic examinations showed the goiters to be hyperplastic. Meanwhile Mackenzie and Mackenzie [35] had observed the development of goiters in animals treated with sulfaguanidine. Their attempts to study the pathogenesis of these goiters led them to work with thiourea and its derivatives. Simultaneously Astwood and his co-workers [36] studied the goitrogenic effects of various sulfonamide compounds, thiourea and related compounds. Both the Mackenzies and the Astwood group discovered that, in spite of the hyperplastic state of the thyroid, the basal metabolic rates of their experimental animals were subnormal. It was not too surprising, therefore, that the administration of iodine compounds had no influence on the development of these goiters. They found, on the other hand, that the simultaneous administration of thyroxin or desiccated thyroid with the goitrogenic substances did prevent the abnormalities which otherwise developed. The examination of the anterior lobe of the pituitary at various stages of their experiments revealed that when the goiters were developing the histologic appearance of the anterior lobe resembled that occurring in the thyroidectomized animal. The removal of the pituitary before the administration of a sulfonamide compound or of thiourea prevented the appearance of the goiters. The simultaneous administration of thyroid hormone with the drugs abolished the histologic changes in the hypophysis as well as in the thyroid glands. Finally, neither sulfonamide compounds nor thiourea had any antagonistic effect as regards thyroid hormone as judged by their simultaneous use in thyroidectomized animals.

The net conclusions from all this evidence can perhaps best be summarized in Astwood's [36] own words:

> The sequence of events is considered to be as follows: Shortly after the drug is administered the organism becomes unable to synthesize thyroid hormone at a normal rate, and the quantity of circulating hormone tends to fall. In response to this deficit an excess of thyrotropin is produced by the pituitary which stimulates the thyroid to hyperplasia and to the release of the normal thyroid hormone stores therein. Within 48 hours of the first administration of the drug these compensatory changes are histologically visible, and for a number of days this mechanism is adequate to maintain the metabolic rate at a normal level. Eventually, however, the store of normal thyroid hormone is exhausted, as evidenced by a complete loss of demonstrable colloid at the end of 7 to 10 days, and as new hormone can be made only at a reduced rate, the metabolic rate falls even though the thyroid hyperplasia is still advancing.[36]

Signs and Symptoms of Hyperthyroidism, According to Immediate Cause

Signs and Symptoms	Direct	Partly Indirect	Indirect
Increased basal metabolic rate	Thyroxin		
Increased excretion of nitrogen	Thyroxin		
Increased organic iodine in blood	Thyroxin		
Increased pulse rate	Thyroxin	Vitamin B deficiency	
Central nervous system effects, tremor, etc.	Thyroxin	Vitamin B deficiency	
Creatinuria	Thyroxin	Vitamin C deficiency	
Increased excretion of calcium	Thyroxin	Vitamin D deficiency	
Loss of weight			Anorexia (vitamin B deficiency)
Loss of glycogen stores			Vitamin B deficiency
Muscular weakness			Pyridoxine deficiency
Disturbance of sex cycle			Vitamin B deficiency
Exophthalmos			Thyrotropic hormone
Goiter			Thyrotropic hormone ± ?



Modified from Williams and Bissell, New Engl. J. Med. 229:97, 1943

Fig. 8.—Data on a case reported by Williams and Bissell [38] (modified from their original chart).

The successful treatment of clinical hyperthyroidism in human beings with thiourea and

33. Kennedy, T. H.: Thioureas as Goitrogenic Substances, Nature, London **150**:233, 1942.

34. Richter, C. P., and Clisby, K. H.: Toxic Effects of Bitter-Tasting Phenylthiocarbomide, Arch. Path. **33**:46 (Jan.) 1942.

35. Mackenzie, C. G., and Mackenzie, J. B.: Effect of Sulfonamides and Thioureas on the Thyroid Gland and Basal Metabolism, Endocrinology **32**:182, 1943.

36. Astwood, E. B.; Sullivan, J.; Bissell, A., and Tyslowitz, R.: Action of Certain Sulfonamides and of Thiourea upon the Function of the Thyroid Gland of the Rat, Endocrinology **32**:210, 1943.

thiouracil, including reduction of the goiter in some instances, has now been reported in some 15 cases by Astwood,[37] by Williams and Bissell [38] and by Himsworth.[39] Figure 8 illustrates a case of Williams and Bissell. It may be seen that in addition to the usual clinical criteria, such as weight and basal metabolic rate, these authors also studied the variations in the total organic iodine in the blood. The latter data corroborated the disappearance of the excessive thyroid hormone.

It is of course too early to make any final estimate of the value of treatment with thiourea in the general run of cases of hyperthyroidism. For one thing, it is not yet clear whether cessation of such treatment will result in a rekindling of the hyperthyroidism in some or in all instances. In a recorded case in which use of the drug was discontinued after a short period of treatment, the hyperthyroidism did recur. It may be that longer periods of treatment will not be followed by recurrence of the disease. There is the possibility that certain types of hyperthyroidism will yield permanent results while others will not. For example, hyperthyroidism which appears during a time of physiologic stress and strain, such as puberty, adolescence or the menopause, may very well be handled by tiding the patient over until the difficult period is past.

It must be emphasized that for the time being the treatment should be regarded as an experimental procedure to be carried on cautiously and with constant supervision and close observation of the patient. A careful watch should be kept for hitherto undetected toxic side reactions of the drugs. In this connection it may be noted that it already appears that thiouracil is preferable to thiourea or other derivatives. And even if no harmful side effects come to light it will be necessary to guard against the dangers of overtreatment. The administration of thiouracil to the point at which a lower than normal basal metabolic rate is obtained involves the possibility of two untoward results, both of them due to the reflex stimulation of excessive secretion of thyrotropic hormone from the anterior lobe of the pituitary. This might cause an increase in the size of the goiter and initiation or exaggeration of exophthalmos. It is probable that both these dangers may be avoided by adding to the treatment the administration of ½ to 1 grain (0.03 to 0.06 Gm.) of desiccated thyroid per day. The administration of thyroid should not be started until the basal metabolic rate has been maintained at or about the normal level for one to two weeks. The patient of Williams and Bissell [38] whose case is illustrated by figure 8 started out with a slight exophthalmos. During three weeks of treatment with thiouracil alone, the exophthalmos and other ocular symptoms increased in severity, while the basal metabolic rate was falling. The addition of first ½ grain (0.03 Gm.) and later 1 grain (0.06 Gm.) of desiccated thyroid per day caused a disappearance of the ocular symptoms without materially affecting the basal metabolic rate.

The facts which have been outlined represent a gratifying amount of progress within a brief period of time in a subject which had seemed almost dormant for many years. They should give particular satisfaction to the physiologist and the internist, who for so long have been faced with an essentially medical problem for which only surgery seemed to offer a practical solution.

37. Astwood, E. B.: Treatment of Hyperthyroidism with Thiourea and Thiouracil, J. A. M. A. **122**:78 (May 8) 1943.

38. Williams, R., and Bissell, J.: Treatment of Hyperthyroidism with Thiouracil, New England J. Med. **229**:97, 1943.

39. Himsworth, H. P.: Thyrotoxicosis Treated with Thiourea, Lancet **2**:465, 1943.

ADRENAL AMYLOIDOSIS

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In the investigation of the problems of generalized amyloidosis we have had ample opportunity to study the development of adrenal amyloidosis. To correlate the clinical signs and symptoms which might be due to adrenal insufficiency with the degree of adrenal amyloidosis and to determine what metabolic studies may be useful in differentiating generalized amyloidosis, adrenal amyloidosis and the underlying disease producing amyloidosis is the purpose of these observations.

MATERIAL AND METHOD

During the past eleven years there were 468 cases of generalized amyloidosis at Sea View Hospital in 449 of which gross and microscopic examinations of the adrenals were made. In all but 7 patients (1 with bronchiectasis, 1 with syphilis and 5 with pulmonary abscesses), the underlying disease was tuberculosis. In 354 (81 per cent) of the patients the adrenal glands showed amyloidosis. Rosenblatt [1] reported adrenal amyloidosis in 40 per cent of his patients with generalized amyloidosis; Bronfin and Guttman,[2] in 78 per cent of theirs. We believe that the percentage would be greater in our series if microscopic sections of all the adrenal glands had been stained with methyl violet. In routine microscopic study of the tissues we have been able to demonstrate deposits of amyloid when it was present in minimal and extensive amounts by staining with hematoxylin and eosin. However, in adrenal glands in which amyloid is present in minute amounts, with the distribution usually limited to the walls of the blood vessels, its presence can be demonstrated only when the tissue is stained with methyl violet (methylrosaniline chloride). Cases in which the latter condition is present are, in any event, of only academic importance.

PATHOLOGIC PICTURE

Amyloidosis of the adrenal glands was always part of a generalized process in which the spleen was involved in all instances, the liver and kidneys in 84.4 per cent and 83.1 per cent respectively. Both of the adrenal glands were involved to approximately the same degree, except in those cases in which there was tuberculous involvement limited to one gland.

Gross Appearance of the Adrenal Glands.—There are two constant developments as amyloid degeneration progresses: One is an increase in the consistency of the organ; the other is an increase in its size and weight.

It has often been emphasized that amyloidosis preserves the tissue, maintaining it intact a long time after it has been removed from the body. This is best seen in the adrenal glands when the amyloidosis is moderate or extensive. It is well known that the medullary portion of the adrenal glands usually shows extensive postmortem autolysis. In glands in which amyloid has been deposited to any degree autolysis does not occur and the well preserved cortex and medulla are readily discernible. In glands in which amyloidosis is just beginning, central autolysis takes place and there are no gross changes which would make one suspicious of amyloidosis. The routine application of iodine to the tissue may not reveal any changes or may show faintly staining mahogany-colored pinpoint areas in the region of the zona fasciculata.

As the amyloid process continues characteristic changes develop. The adrenal gland becomes firmer, and is waxy in appearance. Both the cortex and the medulla are increased in size, and the organ itself is increased in volume. The application of iodine reveals mahogany-colored areas, more evident in the cortex than in the medulla, with the greatest deposition in the zona fasciculata.

The adrenal gland was found to be an excellent organ in which to study progressive deposition of amyloid. The combined weight of the two adrenal glands was recorded in 98 instances. In the evaluation of weights glands were excluded which in addition to amyloidosis had extensive caseation, since the latter alone greatly increases the weight of the gland. The greatest weight recorded in our series was 40 Gm., recorded in 4 cases.

From table 1 the close relationship between the weight of the adrenal glands and the extent of the amyloid deposition is readily observed. The relationship is even closer than that observed

From the Departments of Medicine and Pathology, Sea View Hospital.

1. Rosenblatt, M. B.: The Clinical Manifestations of Amyloidosis, Ann. Int. Med. **8**:678-689 (Dec.) 1934.

2. Bronfin, I. D., and Guttman, P. H.: Amyloid Degeneration of the Adrenals as a Factor in Producing Symptoms of Addison's Disease in Chronic Pulmonary Tuberculosis, Am. Rev. Tuberc. **31**:1-24 (Jan.) 1935.

for the liver, in which progressive deposition of amyloid likewise produces progressive increase in weight.

Microscopic Appearance of the Adrenal Gland.—The first depositions occur in the zona fasciculata and in the early stages can be recognized only when stained with methyl violet. The amyloid is laid down in the interstitial tissue in the region of the capillaries, and as the deposition increases it is recognizable in sections stained with hematoxylin and eosin as pink homogeneous clumps. This stage, which we have designated minimal amyloidosis, occurred in 153 cases (34.1 per cent).

The amyloid zones in the cortex increase in number as the process continues, and in many areas they fuse, while at the same time depositions appear in the medulla. As the amount of amyloid deposited in the interstitial tissue of the cortex increases, it produces pressure atrophy of the neighboring epithelial cells. Thus as the process continues the compressed epithelial cells show all stages of atrophy to complete disappearance. In advanced amyloidosis large portions of the adrenal cortex, particularly the zona fasciculata, disappear, and the areas are almost completely occupied by the pink homogeneous zones. The zona glomerulosa is the last layer to be involved by amyloid infiltration, and in the late stages of the process, when amyloid has replaced the greater portion of the other cortical layers, there may still be only minimal deposits in this zone. This peculiarity was also observed by Bronfin and Guttman.[2] Usually, when amyloid degeneration has developed to a moderate degree in the zona fasciculata, it also appears in the zona reticularis and the medulla, with minimal deposits in the zona glomerulosa. This stage, which we have designated moderate amyloidosis, occurred in 111 cases (26.1 per cent).

TABLE 1.—*Weight of the Adrenal Glands in Relation to the Extent of Amyloidosis*

Extent of Amyloidosis	Weight of Adrenal Glands		
	Normal	Increased	Total
Minimal	24	10	34
Moderate	24	9	33
Severe	17	14	31
Total	65	33	98

In a number of instances deposition was so extensive that almost the entire adrenal parenchyma was replaced by amyloid. There was an extreme increase in the size of the gland, and it had a diffuse pink homogeneous appearance with occasional remnants of epithelial cells. Severe amyloidosis occurred in 94 of our cases (20.9 per cent).

CLINICAL MANIFESTATIONS

In none of the 354 cases in our series was there indubitable clinical evidence of adrenal cortical hypofunction (Addison's disease). The rarity of amyloidosis as a cause of adrenal cortical hypofunction was also observed by Guttman [3] in his analysis of 566 cases reported in the literature, in only 7 of which (1.73 per cent) was the lesion in the adrenal gland the result of amyloidosis. Since his report covering the literature up to 1929 was published, occasional isolated studies of cases have appeared, notably Klein's,[4] in which adrenal amyloidosis associated with amyloid nephrosis was reported.

Bronfin and Guttman [2] suggested three possible explanations for the extreme rarity of adrenal cortical hypofunction due to amyloid infiltration: (1) that sufficient cortical tissue remains to prevent symptoms of the disease; (2) that the medulla, which is spared of amyloid infiltration, prevents the complete development of Addison's disease, and (3) that symptoms of Addison's disease may be masked in many instances by the underlying pathologic condition, usually extensive chronic pulmonary tuberculosis.

It is this last possibility which, with the help of observations of metabolic conditions, we have attempted to clarify. While none of our patients had frank Addison's disease, all of the symptoms except pigmentation have appeared in varying degrees both in the patients with generalized amyloidosis and in those with chronic pulmonary tuberculosis. In the following discussion the clinical symptoms and chemical disturbances attributed to adrenal insufficiency are discussed, although, since the medulla is involved in only late stages, we have actually studied cortical rather than adrenal insufficiency. In general, the cases in which amyloidosis of the adrenals was minimal or absent have been used as controls to evaluate the changes in those in which there was moderate or severe adrenal amyloidosis. The degree of amyloidosis, according to the criteria previously established, was determined in each case before the clinical and laboratory data were recorded. However, since as far as is possible we have correlated the degree of amyloidosis with measurable metabolic changes, rather than with less exact symptoms, the personal factor is probably negligible.

3. Guttman, P.: Addison's Disease, Arch. Path. **10**:742-785 (Nov.); 895-914 (Dec.) 1930.

4. Klein, J. E.: Amyloidosis Complicated by Nephrosis and Addison's Syndrome, M. Rec. **143**: 465-468 (June 3) 1936.

Disturbances in Electrolyte Metabolism.—The decrease in sodium and increase in potassium ions in the blood have been studied extensively and constitute one of the most specific changes in adrenal cortical insufficiency. The salt restriction test of Cutler, Power and Wilder [5] to determine disturbances of the electrolytic balance by calculation of the urinary chlorides under certain standard conditions, including restriction of the amount of salt in the diet and administration of potassium citrate, has proved to be of great diagnostic assistance. This test was performed under the direction of Dr. E. Ornstein [6] for 20 of our patients with generalized amyloidosis, 11 of whom were shown to have severe, 6 moderate, 2 minimal and 1 no adrenal amyloidosis (table 2). There was no tuberculosis of the adrenal glands in any of these patients. One test was discontinued because of the patient's poor pulmonary condition. In other patients varying degrees of hypotension, nausea, vomiting and headache developed, but it was not necessary to discontinue the tests.

TABLE 2.—*Reaction to the Salt Restriction Test for Adrenal Insufficiency* [5] *in Relation to the Extent of Amyloidosis*

Extent of Amyloidosis	Reaction		
	Positive	Equivocal	Negative
Severe	7	2	2
Moderate	2	2	1
Minimal	2	0	0
None	1	0	0

Negative results were obtained for the salt restriction test for 2 patients who showed severe amyloidosis at autopsy, but the tests had been performed two years and fourteen months respectively before death, when the adrenal glands might not have been and probably were not as extensively involved as at autopsy. The results therefore cannot definitely be classified as falsely negative.

Two patients with minimal and 1 with no amyloidosis at autopsy had definite positive reactions to the test. However, these patients had edema due to amyloid nephrosis at the time of the determinations. The conditions of the test (restriction of the amount of salt in the diet and administration of potassium citrate) may have produced diuresis with consequent increased excretion of salt. That is, the increased excretion of sodium chloride may have been due not to adrenal insufficiency but to the therapeutic effects of the diet and the medication on nephrosis. The presence or absence of diuresis at the time of the test was not recorded; so this point cannot be determined definitely. Cutler, Power and Wilder [5] noted a diminution in the volume of urine excreted by patients with adrenal cortical hypofunction under the conditions of the test, but apparently none of their patients had the massive edema which is associated with nephrosis. The discrepancy between these positive results and the conditions observed at autopsy could not have been due to absorption of amyloid in the interval between the test and death since all of the tests were performed within one month before death.

Although the number of patients for whom the salt restriction test was performed is small, the results indicate that the test has some value but that the presence of edema and the diuretic effects of the procedures used should probably be considered in evaluating results. Except in the 3 cases in which false positive results were obtained, there was a fairly close correlation between the amount of chloride excreted and the extent of amyloidosis. Other physiologic changes in the 7 patients with severe adrenal amyloidosis and positive reactions to the salt restriction test are discussed in the sections which follow. None of these patients showed pigmentation.

The chloride content of the blood was determined, as sodium chloride, in 25 cases. This number excludes cases in which the determination was made more than four months before the patient's death, during the course of a test of adrenal cortical function or during an episode of uremia with elevation of the nonprotein nitrogen content of the blood. As was expected, unless the chloride ion is determined separately, there is no correlation between the extent of the adrenal amyloidosis and the level of the blood chloride. In all but 1 of the 25 cases the values were normal. In the exceptional case the Cutler salt restriction test was not performed. Five of the 7 patients with positive reactions to the salt restriction test and severe amyloidosis had between 350 and 400 mg. of sodium chloride per hundred cubic centimeters of blood at the time of the test.

Arterial Hypotension.—In table 3 the blood pressures listed represent the average range during the last six months of life. Terminal pressures, determined during the last week to ten days of life, postoperative pressures or pressures determined during tests of adrenal cortical function are excluded.

5. Cutler, H. H.; Power, M. H., and Wilder, R. M.: Concentrations of Chloride, Sodium and Potassium in Urine and Blood, J. A. M. A. **111**:117-122 (July 9) 1938.

6. Ornstein, E. A.: Adrenocortical Insufficiency in Amyloid Disease, Quart. Bull., Sea View Hosp. **5**:21-26 (Oct.) 1939.

Blood pressures of 100 or less systolic and 70 or less diastolic occurred in 14 (36.8 per cent) of 38 persons with severe amyloidosis, in 17 (45.9 per cent) of 37 with moderate amyloidosis, in 13 (24.5 per cent) of 53 with minimal amyloidosis, in 13 (37.1 per cent) of 35 without amyloidosis and in 7 (50 per cent) of 14 with amyloidosis and tuberculosis.

If the patients with minimal amyloidosis or none are grouped together as controls, and those with severe amyloidosis or with amyloidosis and tuberculosis are grouped together, then one finds that 26 (29.5 per cent) of the 88 controls and 21 (40.4 per cent) of the patients with severe pathologic changes in the adrenal glands had low blood pressure. Of the 7 persons with positive reactions to the salt restriction tests and severe amyloidosis, 4 had normal pressures; in 2 of the 4 the blood pressure fell during the tests. Three had low pressures, and in them the pressure fell further during the test.

Many patients with severe amyloidosis showed evidence of uremia or of preuremic conditions. This condition evidently did not influence the general level of the blood pressure, however, for the blood pressures of these patients were distributed about equally in the low, average and high groups.

Low blood pressure in the presence of amyloidosis and tuberculosis is therefore an indication, but only a fair one, that the adrenal glands are extensively involved with amyloidosis and/or tuberculosis. The pulmonary disease alone, however, is able to produce hypotension, and such hypotension can only be considered an indication for further investigation of possible adrenal insufficiency.

Renal Dysfunction.—Disturbances in renal function were difficult to evaluate, since many of our patients with amyloidosis of the adrenal glands had amyloid nephrosis. Table 4 presents the relation of amyloidosis to a single index of the efficiency of the kidneys, the amount of nonprotein nitrogen in the blood. The determinations were all made within three months of death, in each instance the last determination being the one recorded. Of the 37 patients with elevated values, all but 4 died with uremia or were preuremic. In these 4, while microscopic examination of the renal tissue indicated that an apparently sufficient number of normal glomeruli remained for adequate function, a moderate number of glomeruli were replaced by amyloid. Of the 7 patients with positive reactions to the salt restriction test and severe amyloidosis, all except 3 who died with uremia had normal amounts of nonprotein nitrogen.

Tests of concentration of urine, urea clearance and urinary excretion of phenolsulfonphthalein were also made and yielded similar results. The abnormalities which occurred were so closely related to the extensive involvement of the kidneys that any effect which the adrenals might have had on renal function was overshadowed by the renal amyloidosis.

TABLE 3.—*Blood Pressure in Relation to the Extent of Amyloidosis*

	Blood Pressure							
Extent of Amyloidosis	Systolic / Diastolic	80 or less / 50 or less	85 / 40 to 50	90 to 95 / 50 to 60	95 to 100 / 60 to 70	105 to 110 / 70 to 80	115 to 130 / 80 to 90	140 to 150 / 100	150 or over / 110 or over
Severe		1	0	6	7	13	10	0	1
Moderate		1	0	2	14	10	9	1	0
Minimal		1	2	1	9	19	15	4	2
None		0	0	1	12	11	9	2	0
Amyloidosis plus tuberculosis		1	0	1	5	3	3	1	0

TABLE 4.—*Nonprotein Nitrogen of the Blood in Relation to Amyloidosis*

	Nonprotein Nitrogen, Mg./100 Cc. of Blood			
Extent of Amyloidosis	25 to 40	41 to 50	51 to 100	Over 100
Minimal	41	3 (2)*	5 (5)	5 (5)
Moderate	41	2 (1)	4 (3)	2 (2)
Severe	32 (1)	2 (2)	4 (4)	9 (9)
None	29	0	0	0

* Figures in parentheses indicate the number of patients who died with uremia or preuremia.

Disturbances of Carbohydrate Metabolism.—Seven tests of dextrose tolerance were done. There was no correlation between the extent of amyloidosis and the type of curve obtained. None of the patients tested was diabetic, but most of them were in the older age group. Three of the 5 persons with severe amyloidosis of the adrenal glands had curves typical of latent diabetes, and 1 had a normal curve. Here also the effects of pathologic changes in another organ, the liver, must be considered. Even if disturbances in the storage of glycogen could be determined, it would be impossible to decide how much was due to pathologic changes in the liver and how much to that in the adrenal glands.

Adynamia.—The outstanding subjective symptom of adrenal cortical insufficiency, adynamia,

we have found completely unreliable as an indication of the degree of adrenal amyloidosis in this group of patients, partially because it is unmeasurable and partially because it is also one of the outstanding symptoms of chronic pulmonary tuberculosis. According to Loeb,[7] adynamia in adrenal cortical insufficiency is probably chiefly dependent on disturbances in the carbohydrate metabolism and to a lesser extent on disturbances in the electrolyte balance. Both these factors we have already considered.

Fever.—In table 5 the general range of temperature during the last three to four months of life is recorded. Terminal temperatures are excluded, since there would otherwise be a distortion not necessarily dependent on adrenal amyloidosis.

The preponderance of temperatures slightly above normal in patients with severe amyloidosis

TABLE 5.—*Temperature in Relation to Amyloidosis*

Extent of Amyloidosis	Sub-normal	Normal	Slightly Increased	High	Total
Minimal	0	1 (0.9%)	27 (22.1%)	94 (77%)	122
Moderate	1 (1.1%)	2 (2.2%)	31 (34.1%)	57 (62.6%)	91
Severe	1 (1.4%)	9 (12.7%)	36 (50.7%)	25 (35.2%)	71
None	0	0	12 (17.2%)	58 (82.8%)	70
Amyloidosis plus tuberculosis	0	1 (4.1%)	4 (16.7%)	19 (79.2%)	24

is striking; half of the persons with severe adrenal amyloidosis had temperatures falling in this range, while only one third were hyperpyrexic. Since the tuberculous disease was similar in all groups, this is significant. Of the persons with extensive involvement of the adrenal glands (those with amyloidosis and tuberculosis) over three-fourths were hyperpyrexic.

The low figure of 35.2 per cent for the persons with severe amyloidosis and with hyperpyrexia may be slightly influenced by the fact that, in general, the greater the extent of amyloidosis the longer the duration of the disease and therefore the less acute the condition. Twenty-five persons with severe amyloidosis (35.3 per cent) had had the disease five years or more. However, 35 patients with severe amyloidosis of the adrenal glands (49.3 per cent) had had the disease two to four years, which is approximately the same duration as in those with minimal, moderate or no adrenal amyloidosis.

The underlying disease is the most important factor in the temperature curve, but its pyrexic effect apparently can be mitigated by the presence of severe amyloidosis of the adrenal glands. Therefore, when the disease is such that one would expect fever but the patient's temperature is normal or only slightly elevated, investigation for adrenal amyloidosis is indicated. There is, moreover, a closer relationship between temperature and degree of adrenal amyloidosis than there is between blood pressure and the extent of amyloidosis. Of the 7 patients with positive reactions to Cutler's salt restriction test and severe amyloidosis 4 had low grade fever, 1 had a normal temperature, 1 was hyperpyrexic and for 1 no temperature chart was available for study.

Recent charts of both blood pressure and temperature were not available for sufficient persons to determine exactly whether or not a correlation exists between the two. In general, patients with low blood pressure and minimal amyloidosis were hyperpyrexic, while those with low blood pressure and severe amyloidosis had low grade fever.

Gastrointestinal Disturbance.—Gastrointestinal disturbance was by far the commonest symptom of adrenal cortical insufficiency in our patients but one which could be explained equally well on the basis of renal amyloidosis or of intestinal tuberculosis.

Of the 7 patients with positive reactions to the salt restriction test and severe amyloidosis, 4 had no gastrointestinal symptoms other than at the time of the test and 3 had symptoms which may have been due to another disease.

There were 2 cases of minimal and 1 of severe amyloidosis in which, in addition to containing deposits of amyloid, the adrenal glands were caseous. Since in these cases there were no other diseases that could account for the gastrointestinal symptoms, the latter may have been due to adrenal insufficiency. In 1 of these cases the blood pressure was low, ranging below 100 mm. systolic and 70 diastolic and the temperature was slightly elevated. In the other 2 also the temperature was slightly elevated but the blood pressure had not been determined early enough to be significant.

CONCLUSIONS

Since amyloidosis produces extensive anatomic changes in the adrenal glands, not only by replacement of normal cortical tissue but by associated atrophy due to pressure, it is likely that some degree of adrenal insufficiency is produced, with or without evidence of frank Addison's disease. We have reviewed the clinical and physio-

7. Loeb, R.: Adrenal Cortex Insufficiency, J. A. M. A. **116**:2495-2500 (May 31) 1941.

logic phenomena usually associated with adrenal amyloidosis in order to determine whether or not any criteria exist which may be helpful in determining to what extent these phenomena are due to adrenal insufficiency and to what extent to the underlying disease. Although we have been able to find no single criterion which can definitely settle this point, certain factors have been found useful. In the order of their relative importance and probability, the evidences of adrenal insufficiency are as follows: a positive or equivocal reaction to the salt restriction test in the absence of edema; low grade fever or normal temperature in the presence of active infection and low blood pressure. Changes in renal function and carbohydrate metabolism, adynamia and gastrointestinal disturbances are of little or no assistance as differential criteria.

Since a certain degree of adrenal cortical insufficiency probably exists in patients with severe amyloidosis and possibly some in those with moderate amyloidosis, an effort should be made to discover the severity of this insufficiency in every person with generalized amyloidosis. If evidence of even mild insufficiency exists, appropriate therapeutic measures should be instituted. This is particularly true, as Ornstein [6] has pointed out, in cases in which major operations are contemplated.

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Progress in Internal Medicine

SYPHILIS

REVIEW OF THE RECENT LITERATURE

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BALTIMORE

The material for this article has been selected from publications which have appeared from July 1943 to July 1944. As in previous reviews,[1] it has been necessary rigidly to select material, excluding comparative serologic studies and most case reports. The number of European journals available for review is negligible. Because the war has focused attention on the prevention of syphilis, especially among the armed forces, again this year there are many articles on control of venereal disease.

TREPONEMA PALLIDUM

Cultivation of Treponema Pallidum.—Previous attempts have been made to grow Treponema pallidum on the chorioallantoic membrane of the hen's egg. The results of these experiments were not convincing. Wile and Johnson [2] report another such experiment.

Three dozen eggs were incubated at 38 to 39 C. for ten to thirteen days. After it was determined by candling that the eggs contained living embryos, they were inoculated with an emulsion of rabbit testicular chancre containing one to three actively motile spirochetes of the Nichols strain per dark field. Control rabbits were inoculated intratesticularly with the same emulsion. The eggs were then placed in an incubator at 35 C. for eight days. At the end of this time, 8 eggs were selected at random and opened. The chorioallantoic membranes, together with the hearts, livers and breast muscles of the 8 embryos, were pooled and emulsified. This material failed to reveal spirochetes on dark field examination. To test for virulence, this emulsion was injected into the testes of 2 rabbits which had negative serologic reactions. At weekly intervals the rabbits were examined for induration and swelling of the testicles, and at approximately monthly intervals serologic tests for syphilis were repeated. If these examinations showed physical or serologic evidence of syphilitic infection, the testicle was then aspirated and the material so obtained examined with the dark field microscope. If this examination disclosed T. pallidum, a testicular or lymph node transfer was performed to confirm infectiousness. If no serologic or physical evidence of syphilis was demonstrated by examination of the rabbit at the end of one hundred and twenty days after inoculation with the emulsified egg, the rabbit was reinoculated with an emulsion of rabbit testes that had been found to contain the treponemes by dark field examination. In all rabbits so inoculated physical and serologic evidence of syphilis developed.

Five such sets of experiments were performed. Of these, one was successful. Two rabbits, inoculated from the same dark field–negative chorioallantoic membranes and chick embryo tissue, showed serologic and physical evidence of syphilitic infection. The blood serum of both of these rabbits gave positive reactions at the end of seventy-seven days. The titer in each case was 128 Kahn units. Testicular chancres were present, and in both instances T. pallidum was found. The method deserves further study.

Motility.—Cares [3] discusses lucidly the technic of dark field examination. His major purpose is,

From the United States Public Health Service and the Johns Hopkins University Venereal Disease Research and Post-Graduate Training Center.

1. (*a*) Moore, J. E.: Syphilis: A Review of the Recent Literature, Arch. Int. Med. **56**:1015 (Nov.) 1935. (*b*) Padget, P., and Moore, J. E.: Syphilis: A Review of the Recent Literature, ibid. **58**:901 (Nov.) 1936; (*c*) **60**:887 (Nov.) 1937. (*d*) Padget, P.; Sullivan, M., and Moore, J. E.: Syphilis: A Review of the Recent Literature, ibid. **62**:1029 (Dec.) 1938. (*e*) Moore, J. E., and Mohr, C. F.: Syphilis: A Review of the Recent Literature, ibid. **64**:1053 (Nov.) 1939. (*f*) Mohr, C. F.; Padget, P., and Moore, J. E.: Syphilis: A Review of the Recent Literature, ibid. **66**:1112 (Nov.) 1940. (*g*) Mohr, C. F.; Padget, P.; Hahn, R., and Moore, J. E.: Syphilis: A Review of the Recent Literature, ibid. **69**: 470 (March) 1942. (*h*) Reynolds, F. W.; Mohr, C. F., and Moore, J. E.: Syphilis: A Review of the Recent Literature, ibid. **70**:836 (Nov.) 1942; (*i*) Syphilis: A Review of the Recent Literature, ibid. **72**:635 (Nov.) 1943.

2. Wile, U. J., and Johnson, S. A. M.: Further Study of the Chick Embryo as a Culture Medium for the Spirochaeta Pallida, Am. J. Syph., Gonor. & Ven. Dis. **28**:187 (March) 1944.

3. Cares, R.: Dark-Field Diagnosis of Penile Lesions: Differential Motility Characteristics of Treponema Pallidum, J. Lab. & Clin. Med. **29**:82 (Jan.) 1944.

however, to describe the differential motility characteristics of T. pallidum. He discusses separately the physical characteristics of locomotion and those related to changes in shape or outline of the organism. He defines true locomotion (motility) as any motion which changes the spatial relation of the organism to a fixed point in the field of view. There are two types of locomotion, rotation and propulsion. The rotation of T. pallidum on its longitudinal axis may be moderately sluggish but never reaches the velocity of Spirochaeta refringens or Spirochaeta phagedenis. The propulsion of T. pallidum (that is, its forward or backward movement) is slow, intermittent and frequently reversible. The motion is never fitful, spasmodic or "darting," as seen in refringens and phagedenis spirochetes.

The physical characteristics of shape or outline are described by the author as protean. Flexion or angulation is a constant feature, and almost a diagnostic one. The point of bending may be anywhere along its length. The longer arm is stationary, while the shorter arm sways and swings leisurely. Buckling is almost as frequent as angulation, and resembles precisely the reaction of a coil spring being forcibly compressed by end pressure. Undulation is described as quivering, vibration or fluctuation similar to that of a magnetic compass needle on its center bearing. Other spirochetes show more suppleness or "spineless" flexibility to the axial body, a good differential point. Expansion and compression of the coils are frequent but not diagnostic, being seen in other organisms as well. When T. pallidum is unusually motile, looping of the axis to form an eclipse or narrow-throated U is not infrequent. One pole remains fixed; the other pole arcs slowly to meet in a closed or open loop. While in this state the whole loop may slowly rotate like a ring transfixed on a rotating pole. It may give the appearance of a "tumbling" or end over end movement. The author has not observed looping in other organisms. It is evident that T. pallidum has protean motion far above any other spirochetal form encountered. A point to be emphasized is the relatively slow rate of motility in any of the phases that have been listed.

T. Pallidum under the Electron Microscope.—Studies with the electron microscope [4] were made of the Nichols-Hough, Kroò, and Reiter culture strains of T. pallidum of spirochetes of the virulent Nichols-Hough strain from a rabbit syphiloma and of cultured strains of T. macrodentium and T. microdentium. The periplast enclosing the inner protoplasm of the treponemes, the flagella, the granules, the attachment of dense spheroid bodies and the mode of division are described; but no attempt is made to distinguish the cultured from the noncultured forms by these characteristics.

Viability in Stored Plasma.—Reports of the past three or four years indicated that T. pallidum seeded in human or rabbit plasma remained viable for only forty-eight to seventy-two hours, rarely for ninety-six hours. Selbie,[5] however, found that rabbit plasma inoculated with T. pallidum from minced testicular chancres and stored at 5 C. remained infective for six days. To account for the longer time for which organisms remained viable in this experiment, he offers the explanation of a larger inoculum. Another factor which may account for differences in survival time is the presence of tissue, which may aid in maintaining virulence. Six days is the maximum time that T. pallidum has been observed to survive in stored plasma even under optimum conditions. The fewer organisms and the absence of tissue in stored human syphilitic plasma minimize the risk of its infectivity.

EXPERIMENTAL STUDIES

Morphologic Study of Chancres.—Steiner, Lehman and Chase [6] studied the development of experimental chancres in a series of 20 rabbits at intervals varying from four hours to one hundred and ninety-nine days following subscrotal implantation of chancre tissue. A new technic was developed by which after routine paraffin embedding of tissue blocks several sections were stained alternately with hematoxylin-eosin and by a special silver nitrate reduction method, the latter for the demonstration of spirochetes. The staining methods are detailed in the text. The authors conclude:

1. The first histological response to subscrotal inoculation of chancre tissue was the appearance of heterophilic polymorphonuclear cells. Eighteen hours after inoculation lymphocytes and plasma cells began to appear and to replace the heterophilic cells. With beginning ulceration, usually the twelfth day after inoculation, a regional reappearance of heterophilic cells took place.

2. With the appearance of the new chancre the implant was no longer recognizable; there was, however, a definite topographical relationship between the place of origin and the new chancre and the region of the implant.

4. Mudd, S.; Polevitsky, K., and Anderson, T. F.: Bacterial Morphology as Shown by the Electron Microscope: V. Treponema Pallidum, T. Macrodentium, T. Microdentium, J. Bact. **46**:15 (July) 1943.

5. Selbie, F. R.: Viability of Treponema Pallidum in Stored Plasma, Brit. J. Exper. Path. **24**:150 (Aug.) 1943.

6. Steiner, G.; Lehman, A. J., and Chase, H. F.: Morphological Studies of Primary Chancre in Experimental Syphilis of Rabbits Before and After Treatment, Urol. & Cutan. Rev. **48**:77 (Feb.) 1944.

3. The inflammatory syphilitic reaction was limited to derma, subcutaneous tissue, and tunicae with their muscular layers; only in one instance (199 days after inoculation) did interstitial tissues of the testicular organ proper show inflammatory reaction.

4. As early as six days after inoculation spirochetes were seen in the intact epidermis. These spirochetes penetrated the basement membrane and induced disintegration of epidermal cells leading to ulceration.

5. Spirochetes appeared in the surroundings of the implant as early as four hours after inoculation; they remained at least 12 days in the implant, the implant serving as center of cultivation and receptacle of new generations of spirochetes.

6. There were no morphological characteristics of reproductive forms of spirochetes. However, elongation of organisms with many coils and the arrangement in colonies or in parallel massive strands may be considered an indication of spirochetal reproduction.

7. The disintegration of spirochetes followed a definite pattern; rings and knobs, discs, thick and straight threads, finally irregular polymorphous granular debris. Disintegration occurred in extracellular spaces and led to peculiar vacuolization. In almost every instance regular spirochetes and disintegrating forms were seen concomitantly, either in the same areas or in different regions of the same specimen. This phenomenon has been called heterophasis, indicating the presence of many individual spirochetal generations or a fluctuation in the life spans of one generation. The process of spirochetal disintegration may be considered due to auto-agglomeration.

8. By chance a piece of epidermis was inserted with the implant into the depth of the scrotal pocket; 12 hours after inoculation this piece of isolated epidermis showed invasion by spirochetes, indicating that spirochetes are not strictly parasitic but can invade nonliving tissues.

SERODIAGNOSIS

Biologic False Positive Serologic Reactions for Syphilis.—(*a*) In Spontaneous Malaria: Dawber [7] believes that infectious mononucleosis and malaria are the chief causes of biologic false positive serologic reactions in this country. Unfortunately both of these diseases are common and often are present in a subclinical state. This is particularly true of chronic or latent malaria. It is suggested, but not proved, by the authors that latent malaria may produce positive reactions which may easily be mistaken for evidence of latent syphilis. In a review of all cases of naturally occurring malaria encountered at the United States Marine Hospital, Norfolk, Va., between July 1936 and July 1940, the following facts were established: The number of cases of malaria was 64; in 19 of these reactions to serologic tests for syphilis were positive. In 7 of these 19 cases the condition was diagnosed definitely as syphilis, and in 4, as probably syphilitic. In the remaining 8 the positive serologic reactions were thought to have been false. In all of these there was reversion to seronegativity within ten to eighteen days after the last malarial paroxysm.

(*b*) In Induced Malaria: In the acute stages of malaria, serologic tests for syphilis may give positive reactions in the absence of the latter infection. Moreover, if a syphilitic person with positively reacting serum is inoculated with malaria for therapeutic purposes, a rise in reagin titer may occur during the febrile period. Dorgeloh [8] claims that this increased reagin titer during inoculation malaria is nonspecific. This assertion is based on the finding that the rise in titer occurs when isotonic solution of sodium chloride is used as a diluent in complement fixation tests but not when normal human serum is so used. It is suggested that physical factors rather than true serologic cross reactions due to common antigenic components cause the apparent increase in reagin titer during malarial therapy of syphilitic patients.

Kaplan and Brightman [9] present a slightly different picture. They have studied the effect of induced Plasmodium vivax and Plasmodium malariae malaria on the quantitatively titered New York State Wassermann and standard Kahn tests in patients with latent syphilis and with neurosyphilis. The study group was composed of 33 patients inoculated with P. malariae and 11 inoculated with P. vivax. Serologic studies were performed three times weekly. The authors report:

> Of 33 syphilitic patients treated with quartan malaria, 30 exhibited a progressive fall in the complement-fixation titer for syphilis during the induced malarial infection. Of 16 of the 33 on whom quantitative Kahn tests were performed, 14 exhibited an increase in Kahn units, 1 a decrease, and 1 showed no change at all. In 12 of the 14 who showed an increase in Kahn units a subsequent fall occurred before or just after the last malarial paroxysm.
>
> Among the 11 patients with central nervous system syphilis who were treated with P. vivax infection, 6 showed a progressive fall in complement-fixation to less than 60 per cent of the initial level, and 2 exhibited no significant changes. Three individuals showed a rise in titer although in 1 case this was preceded by a preliminary fall. Flocculation tests were done in 10 of these individuals. An increase in Kahn units was noted in 7 patients, in 4 of whom a subsequent fall occurred. No changes appeared in 3 individuals, 2 of whom had likewise shown no change in complement-fixation titer.

In view of daily laboratory variation in sensitivity of serologic reactions, it is unfortunate

7. Dawber, T. R.: On the Importance of Malaria as a Cause of False Positive Serologic Reactions, Ann. Int. Med. **19**:651 (Oct.) 1943.

8. Dorgeloh, J. R.: The Quantitative Complement Fixation Test for Syphilis in Malaria-Treated Syphilis: Effect of the Diluent, Am. J. Syph., Gonor. & Ven. Dis. **27**:623 (Sept.) 1943.

9. Kaplan, B. I., and Brightman, I. J.: The Course of the Serologic Tests During Therapeutic Malaria in Patients with Syphilis, Am. J. Pub. Health **33**:1073 (Sept.) 1943.

that this factor was not controlled by freezing a portion of each serum and testing the accumulated specimens on a single day.

(*c*) In Other Infections: Kolmer [10] discusses the incidence of false positive reactions in normal persons and in patients with leprosy, malaria, vaccinia and various acute febrile illnesses. Apropos of false positive reactions following smallpox vaccination and in febrile diseases, he reports the results of two experiments on animals. Twelve normal rabbits with previously negative serologic reactions for syphilis were inoculated with virulent vaccine virus. Serologic reactions determined four days and one, two, three and four weeks after the development of vaccinal lesions remained negative in all animals. Similarly, 4 rabbits inoculated intradermally with type I pneumococci, 4 with group A hemolytic streptococci and 4 with Staphylococcus aureus continued to have negative reactions.

In Kolmer's experience, the spirochetal complement fixation test has been of doubtful value as an aid in differentiating false positive and syphilitic serologic reactions. In view of the inadequacy of present day serologic tests, Kolmer believes that the differentiation between syphilis and false positive reactions must be made on a clinical rather than a serologic basis.

Taussig [11] reports 4 instances of biologic false positive serologic reactions for syphilis. The 4 patients had, respectively, malaria, cellulitis of the nose, relapsing fever and pneumonia. The author stresses the importance of delaying antisyphilitic treatment until it is definitely proved that the patient has syphilis.

Boeck's sarcoid has already been listed as a cause of false positive serologic reactions. Reisner [12] presents the clinical and laboratory findings of 35 patients with Boeck's sarcoid, 4 of whom were white and 30 Negro. None of the white patients had positive serologic reactions for syphilis. Of the 30 Negroes, 10 had positive and 2 doubtful reactions. No relationship between sarcoidosis and false positive serologic reactions can be attached to this finding, since the known incidence of syphilis in the average adult Negro population is approximately 25 to 30 per cent. Antisyphilitic therapy had no influence on the clinical manifestations or the course of sarcoid.

10. Kolmer, J. A.: The Problem of Falsely Doubtful and Positive Reactions in the Serology of Syphilis, Am. J. Pub. Health **34**:510 (May) 1944.

11. Taussig, A. E.: On the Persistence of Falsely Positive Serologic Tests for Syphilis in Nonsyphilitic Infections, J. Lab. & Clin. Med. **29**:473 (May) 1944.

12. Reisner, D.: Boeck's Sarcoid and Systemic Sarcoidosis (Besnier-Boeck-Schaumann Disease): A Study of Thirty-Five Cases, Am. Rev. Tuberc. **59**:437 (May) 1944.

Harrison and Osmond [13] believe that since there has been a great increase in the routine use of serologic tests for syphilis and since serologic technics are increasingly sensitive, it has become exceedingly important to emphasize the fact that a positive reaction does not necessarily justify a diagnosis of syphilis. Much error is the result of variation in technic, but "assuming that the technique is satisfactory . . . a positive reaction repeated on two or more specimens, . . . means that the patient is suffering from syphilis provided that the following can be excluded: yaws, leprosy, trypanosomiasis, relapsing fever, malaria, scarlet fever, tropical ulcer, beri-beri, pneumonia, late tuberculosis, diabetes mellitus, enteric fever, scleroderma or malignant tumor . . ." It is also mentioned that glandular fever, vaccinia and other diseases cause false positive reactions.

So-Called Verification Tests.—(*a*) Kahn "Verification Test": Sharing the common anxiety over the growing importance in diagnosis of biologic false positive serologic reactions for syphilis, Kahn, in 1940,[14] described a serologic "verification test" (quotation marks ours). This test, described briefly in this review in November 1942,[15] consisted of subjecting each serum to the standard Kahn test at three different temperatures, 1 C., 37 C. and room temperature. If precipitation was greater at 37 than at 1 C., the result was designated as "syphilitic type"; if greater at 1 C. than at room temperature, as "general biologic type." If no precipitation occurred at any temperature level, the result was called "no antibody" or "negative type." Results not classifiable according to this scheme were called "inconclusive type."

Kahn's own documentation of the experimental evidence permitting these classifications was inadequate. However, the unqualified use of the term "verification test" without quotation marks naturally has led physicians to interpret the word "verification" according to its usual meaning in the English language.[15] This (Webster's "New

13. Harrison, L. W., and Osmond, T. E.: Use and Limitations of the Serum Tests for Syphilis, Brit. J. Ven. Dis. **19**:108 (Sept.) 1943.

14. Kahn, R. L.: A Serologic Verification Test in the Diagnosis of Latent Syphilis, Arch. Dermat. & Syph. **41**:817 (May) 1940.

15. This cloudy use of English is paralleled by the naming of the older Kahn "presumptive test" (quotation marks again ours). Here a physician unfamiliar with the serologic literature and with the guarded meaning which Kahn himself attaches to the word "presumptive" is entitled to believe—and many unfortunately do so believe—that a positive reaction to the "presumptive test" presumes that the patient has syphilis while a negative result presumes that he is free from infection. Neither presumption is, of course, necessarily true.

International Dictionary," 1938) is "act of verifying or state of being verified; confirmation; authentication of truth or accuracy by means of facts, statements, citations, measurements, attendant circumstances, etc." Thus, a physician receiving a report from Kahn of "syphilitic type" or "general biologic type" of "verification reaction" might well feel justified in assuming that the presence or absence of syphilitic infection in the patient is in fact verified.

A systematic study of the applicability of this test was undertaken (with Kahn's collaboration, the serologic work being performed in Kahn's own laboratory), by Chargin and Rein [16] (also previously reviewed in November 1942 [1h]). These workers sent specimens to Kahn from 1,565 patients, some with clinically demonstrable syphilis, some in whom biologic false positive reactions were suspected and some with various other conditions. The results indicated that:

1. Syphilitic persons did not always give the "syphilitic type" of reaction. The type of reaction obtained appeared to vary with the qualitative titer of reagin in the patient's serum. If this was high, a "syphilitic type" of reaction was usually obtained; if it was low, a "general biologic type."

2. Nonsyphilitic persons occasionally gave the "syphilitic type" of reaction.

3. Duplicate specimens from the same patient on the same day or on different days sometimes provided in one specimen a "syphilitic type" and in the other a "general biologic type" reaction.

The data published by Chargin and Rein were a convincing demonstration that Kahn's 1940 technic was neither biologically nor technically sound.

Perhaps persuaded by this study, Kahn now comes forth with two new "verification" technics. The first of these is a so-called "triple quantitative technic" [17]; the second, a so-called "salt dispersibility technic," [18] with which is combined yet another procedure, designated as "method B." (For technical details of these, the reader must consult the original publications.) Serums submitted to Kahn for "verification" tests are subjected to the following procedures: The standard Kahn test and "method B" are carried out. The results are read, and without waiting for a second reading fifteen minutes later the individual tubes are subjected to the "salt dispersibility technic." In the case of weakly positive and negative reactions, the "heat differential technic" is then applied, while in the case of strongly positive reactions, the "triple quantitative technic" is used.

Fortunately for the confused clinician, Kahn does not report to him the objective results of these tests, but gives only his own interpretation thereof. He qualifies the report at the outset with the statement: "The test is applicable to cases in which physicians suspect false positive reactions with complete lack of clinical indications of syphilis," i. e., not only for persons who have positive serologic reactions in the course of or after intercurrent infections but also for at least half of those who probably have latent syphilis. Results are reported as "similar to that obtained in lues [sic]," "general biologic (non-luetic) type of reaction," "negative type," and "inconclusive type." These terms are hedged about (on the report blank) with exceptions and qualifications.

In the text of his latest paper,[18] Kahn says: "The dependability of laboratory results in a given infection is generally determined by the extent to which these results are corroborated by clinical findings of that infection." If this were generally true of serodiagnostic tests for syphilis and if clinical corroboration were generally required *at the time* a positive serologic result was obtained, all such technics would be invalid and none would be necessary. The major usefulness of serodiagnostic tests is not for patients with clinically obvious syphilis, but for those with latent infections. The justification for the diagnosis of latent syphilis in many thousands of cases yearly does not rest on corroboration by anamnestic or clinical data at the time of serologic test but is based instead on epidemiologic experience and the evidence provided by the fact that if the positive serologic reaction is ignored and such patients are untreated, clinical evidence of syphilis *eventually* develops in a considerable number. Kahn continues:

> Negative clinical findings of syphilis represent *the* [italics Kahn's] clinical indicator of false positives, and negative findings cannot have the same value as positive findings. . . . When such [negative] findings corroborate verification results of false positives, the significance of these results immediately becomes fortified, just as the significance of positive Widal reactions becomes fortified by clinical findings of typhoid fever. [Here Kahn's analogy is not clear.] The verification test, like other laboratory methods of an immunologic nature, does not lend itself to mass testing.

16. Chargin, L., and Rein, C. R.: The Kahn Verification Test: An Appraisal of the Test Based on Clinical and Serologic Evidence, Arch. Dermat. & Syph. **44**: 1031 (Dec.) 1941.

17. Kahn, R. L.: A New Verification Method in Serology of Syphilis, Univ. Hosp. Bull., Ann Arbor **8**:45 (June) 1942.

18. Kahn, R. L.: The Verification Test in the Serology of Syphilis, J. Lab. & Clin. Med. **28**:1175 (July) 1943.

In describing the terms used in reporting, Kahn says of the term "general biologic (nonluetic) type":

> This designation . . . is more desirable than the purely negative designation of false positive, since *in addition to indicating absence of syphilis* [italics ours], it indicates the presence of a reaction which in many instances accompanies a pathologic disturbance or biologic imbalance.

The quoted sentence that this technic "does not lend itself to mass testing" seems in contradiction of the quoted definitive statement that a "general biologic type of reaction" indicates "absence of syphilis"; and the latter statement is in still further contradiction of Kahn's own statements that the "general biologic type" of reaction may occur in patients previously treated for syphilis or in a small proportion of known cases of early syphilis. Clinicians can hardly be expected to limit their use of the Kahn "verification test" to cases in which the clinical diagnosis is almost certainly *not* syphilis. They will instead employ it as an aid in deciding whether the patient does or does not have syphilis; in their doing so, specimens will inevitably be submitted on a "mass testing" basis. It is essential, therefore, to know the accomplishment and fallibilities of the test, if any, on precisely such a mass basis.

Neither Kahn himself nor any other of the few serologists who have used Kahn's newer procedures offer any documentation that the claims he makes for his new test are true in actual practice. A study of the present Kahn "verification tests" by independent workers in the same manner as the study of Chargin and Rein, already referred to, is urgently needed.

So much space has been here devoted to this subject because the serologic phenomena noted by Kahn are of the greatest importance for further detailed scientific investigative study. It is clearly of fundamental concern to determine whether the positively reacting substance occurring in certain (perhaps all) normal human beings or produced after intercurrent infections or other stimuli is qualitatively identical with the reagin produced in syphilis. To apply these phenomena to a diagnostic test, designated by so great an authority as Kahn as a "verification test," is, however, distinctly premature on the basis of present information.

Bacteriostasis of Spinal Fluid and Blood Specimens.—Spinal fluid and blood specimens transported through the mail to a central laboratory for serologic testing are not infrequently grossly contaminated with bacteria on arrival. The proportion of such specimens thus rendered unsuitable for testing is appreciable during the entire year but becomes greater during the warm months. Harris and Mahoney [19] describe a simple method of bacteriostasis of spinal fluid specimens. Of all compounds surveyed, merthiolate (sodium ethyl mercurithiosalicylate) was found to be most satisfactory.

Into tubes used for the collection of spinal fluid is pipetted 0.1 cc. of a 1 per cent aqueous solution of merthiolate. The tubes are then placed in a vacuum desiccator over calcium chloride at room temperature and the solution evaporated to dryness. The process usually requires forty-eight hours. After removal from the desiccator the tubes are stoppered with paraffined corks, and they may be stored for several months if kept in the dark. The concentration of merthiolate obtained when 2 to 8 cc. of spinal fluid is added to these tubes has not been found to be sufficient to influence any of the standard serologic tests for syphilis. A series of approximately 500 spinal fluids tested before and after the addition of merthiolate showed no appreciable alteration in the results.

Crawford and Hertert [20] observed that serologic tests on serums from patients under sulfanilamide therapy rarely were unsatisfactory. They therefore tested sulfanilamide as a bacteriostatic preservative. Containers were prepared by pipetting 0.2 cc. of a 2 per cent solution of sulfanilamide in 95 per cent alcohol into previously sterilized tubes and drying with low heat. In observations involving over 3,000 serums tested with and without the sulfanilamide preservative, the percentage of unsatisfactory specimens was reduced from 22 to 1.5. The validity of serologic tests was not impaired by the addition of sulfanilamide to the specimen.

Control for Colloidal Gold Test.—In carrying out the colloidal gold test on spinal fluids, it is necessary to have a known positively reacting control. Because of the difficulty in obtaining quantities of spinal fluid with a strongly positive reaction for use in the laboratory, Bossak and his collaborators [21] undertook to produce a stable globulin solution which would serve as a substitute. Two methods of preparing such globulin solutions are presented, one utilizing glycerinated jack bean meal extract and the other employing dialyzed human blood serum. In proper dilution,

19. Harris, A., and Mahoney, J. F.: Merthiolate as an Effective Bacteriostatic Agent in Spinal Fluid Specimens, Ven. Dis. Inform. **25**:46 (Feb.) 1944.

20. Crawford, J. P., and Hertert, L. D.: Preservation of Wassermann Sera by Means of Sulfanilamide, Mil. Surgeon **93**:274 (Sept.) 1943.

21. Bossak, H. N.; Rosenberg, A. A., and Harris, A.: Substitutes for Spinal Fluids as Colloidal Gold Controls, Ven. Dis. Inform. **24**:194 (July) 1943.

either of these globulin solutions may be used as a substitute for spinal fluid with a strong, "dementia paralytica type" reaction to guide the adjustment of colloidal gold solutions to standard reactivity.

VENEREAL DISEASE AND THE WAR

Civilian Aspects.—(*a*) Prevalence: A statistical study made by officers of the United States Public Health Service [22] indicates that there was a rise of 11 per cent in newly reported cases of gonorrhea among civilians in the United States in the period from July through December 1943. There were 158,000 new cases reported during that time, as compared with 137,000 for the corresponding period in the previous year. In this same period, newly reported cases of syphilis decreased 16 per cent, dropping from 290,000 to 245,0000. Although it is recognized that reporting of venereal diseases in the United States is not complete or adequate, these figures offer basis for comparison. The importance of venereal diseases as a cause of disability is indicated by the fact that 861,000 cases of syphilis and gonorrhea were reported during 1943, which is 70 per cent more than the combined total of cases of diphtheria, malaria, meningitis, pneumonia, poliomyelitis, scarlet fever, smallpox, tuberculosis, typhoid, paratyphoid and typhus.

Vonderlehr and Usilton [23] present the results of a second survey of the number of patients with early syphilis reporting for the first time to private physicians and to venereal disease clinics. The first such survey, in 1938, led to the conclusion that the chance of an individual in the United States acquiring syphilis by the age of 50 years was 1 in 10. The authors emphasize that the oft quoted statement "Syphilis strikes one out of every ten adults" meant not that 10 per cent of the population had syphilis but that in any representative sample of persons born alive in a given year and surviving into each successive age group, one tenth of the total would have acquired syphilis within fifty years, if the attack rates of 1936 and 1937 prevailed. It is further emphasized that the 1 in 10 chance found in the former survey referred to the population as a whole and not to any selected group. The present survey, made in 1939 to 1941, indicates that the chance of acquiring syphilis had dropped from 1 in 10 by the age of 50 years to 1 in 15, a one-third decline. The authors attribute this decline to the accelerated preventive and control program which has been in progress since 1936. Further surveys will be deferred until after the war. Although changes in prevalence rate since 1941 are not known, the authors believe, on the basis of incomplete data, that under conditions of mobilization there has been an increase in the civilian rate of attack.

(*b*) Follow-up of Selective Service Registrants: All Selective Service registrants called up for physical examination have had serologic tests for syphilis. Kresge [24] relates the procedure of follow-up in Philadelphia. Soon after the adoption of the Selective Service Act, in November 1940, the Institute for the Control of Syphilis, Hospital of the University of Pennsylvania, assumed responsibility for the immediate supervision of the follow-up of those registrants in Philadelphia who had positive or doubtful serologic reactions for syphilis. Data obtained on the prevalence of syphilis among selectees showed the Pennsylvania incidence to be that of an average industrial state, but the Philadelphia rate, of 41.7 per thousand, proved the highest of any city with over a million population. Subsequent analyses have shown this to be due, at least in part, to the fact that Negroes make up 12.9 per cent of the total population of the city.

When the names of registrants for follow-up were received by the institute from the health department, a form letter was sent to the registrant, informing him that the blood test made when he was examined for Selective Service showed that he might need treatment. The letter advised him to report to a private physician or a clinic of his choice and gave the address and hours of a clinic near his home. This letter provided space for the physician to indicate that the registrant had reported and the date treatment was started, or if treatment was not begun, why. A stamped, self-addressed envelope was enclosed to facilitate the return of these forms.

Of 4,661 letters advising medical care, 1,916 (41.1 per cent) were returned with the record satisfactorily completed. In many of these satisfactory cases, the registrant visited the institute worker. Some of the men wished assistance in making plans for treatment; some who had already received treatment questioned the necessity of further medical care; others desired more information about the test.

If nothing was heard from the registrant or his physician within two weeks, a second letter was sent. An estimated 17.4 per cent of the

22. Venereal Disease Rates in the United States, Current Comment, J. A. M. A. **125**:214 (May 20) 1944.

23. Vonderlehr, R. A., and Usilton, L. J.: The Extent of the Syphilis Problem at the Beginning of World War II, Am. J. Syph., Gonor. & Ven. Dis. **27**:686 (Nov.) 1943.

24. Kresge, A. M.: A Technic of Follow-Up of Selective Service Registrants with Syphilis in Philadelphia, Ven. Dis. Inform. **25**:167 (June) 1944.

total number responded satisfactorily to this second letter. If no response was obtained within another two weeks, the registrant was visited at his home. If the registrant was not at home, a note in a plain sealed envelope was left for him. This note asked the registrant to notify the institute worker at once regarding his plans for medical care, in order that his local board could be notified. In some instances a second home visit was made. If this effort was unsuccessful, the record was closed as an unsatisfactory disposition.

During the two years from July 1, 1941 to June 30, 1943, a total of 4,861 cases were investigated. Of this number, 3,504 (72 per cent) were satisfactorily closed as to follow-up. As a direct result of the follow-up work, 48 per cent of the total number of men investigated were placed under treatment, and 16.3 per cent were found to be already under treatment. The time of beginning treatment was unknown in 7.6 per cent of the cases.

An attempt has been made to estimate the cost involved in placing one registrant under treatment. The approximate expense in salaries of clerical assistants, public health nurses and lay follow-up workers, carfare, stationery, postage and telephone amounted to $7,449.36 for the two years, giving an average cost of $3.18 for placing one registrant under treatment. This figure is the cost only for patients known definitely to have begun treatment as a result of follow-up efforts and does not include the 16.3 per cent found to be already under treatment or the 7.6 per cent for whom the time of beginning treatment is unknown. If the cases of these men are included as satisfactory dispositions, the total cost per patient was $2.13.

(*c*) Syphilis Control: Heller [25] states that wartime venereal disease control requires a two front attack. The tendency toward increase in commercialized sex activity must be opposed. This may be termed social control. Simultaneously, medical control must be intensified; persons in infectious stages must be located, and placed and kept under treatment. Public education and prophylaxis are secondary but important measures. The extent of the problem is indicated by Selective Service data. Among the first 2,000,000 men examined, 47.7 in every thousand aged 21 to 35 had serologic evidence of infection; among Negro men the rate was 272 per thousand and among white men 23.5. The combined rate in the southern portion of the country was more than four times that in the northern.

Medical control has been strengthened as evidenced by the increase in the number of public clinics, in the admission rate for syphilis, in the doses of arsenicals distributed and in the budget appropriations. Social control has been strengthened by an eight point agreement which includes repression of prostitution and sexual promiscuity in areas where armed forces or industrial workers are concentrated. Epidemiologic progress is evident in that there are now at least 8,000 health department employees engaged in work in the field of venereal disease epidemiology.

By July 1942, as a result of the program of the Social Protection Division of the Federal Security Agency in cooperation with local law agencies, more than 300 communities had begun enforcing antiprostitution laws. This increased efficacy of repression of prostitution, coupled with intensified efforts to locate infectious persons, augmented the problem of where to isolate and treat. The Federal government and some state health departments have provided facilities in which certain infectious persons can be isolated and treated under shortened schedules.

(*d*) Control: Parran [26] states that education is an important arm of venereal disease control. Close working relationships have been developed between the Public Health Service and the armed forces, as well as between this service and other federal and numerous nongovernmental agencies. The educational staff of the service has been expanded, informational materials increased and consultation and advisory services established. There has been a steady increase of educational activity by the states and larger cities. The Public Health Service helps the states in training personnel for special educational effort. A well planned program of venereal disease education at state and local levels can be created with the services of one or more full time people who have the ability to plan and execute programs.

Parran calls attention to various other activities of the Public Health Service in education on venereal disease. These include preparation of motion pictures, booklets and posters and of manuals for the health officer, educational efforts in industry, and increased use of Negro workers. An intensified national campaign of information and education has been planned for 1944. This program will consist of articles and sponsored advertisements in magazines of national circulation,

25. Heller, J. R., Jr.: Syphilis Control in War Time, South. M. J. **37**:219 (April) 1944.

26. Parran, T.: The Current Status of Venereal Disease Control Education, J. Social Hyg. **30**:1 (Jan.) 1944.

display of films on venereal disease in theaters, and articles, news stories, photographs, and editorials in the press. It is hoped that radio will also be included.

Doak [27] describes the Venereal Disease Education Institute, a project located in Raleigh, N. C., sponsored by the United States Public Health Service, the North Carolina State Board of Health and the Zachary Smith Reynolds Foundation. The primary purpose of this institute is to provide a constant flow of new and effective educational materials to any agency engaged in venereal disease control. Along with the production of these educational materials (posters, booklets, kodachrome slide sets, a guide for clinic interviewers, advertisements), a program of survey and evaluation of the results achieved has been embarked on.

Military Aspects.—(*a*) Incidence in Inductees: Still and Greenwald [28] report results of a syphilis survey on a group of newly inducted soldiers at Fort Belvoir. This study was undertaken because it was felt that a number of soldiers who were being inducted had active venereal disease, which had been overlooked by induction boards. Included in the survey were three battalions, two white and one Negro, of soldiers all of whom had been classified 1-A and presumably were free of venereal disease at the time of induction. The survey consisted of (*a*) investigation of the history for manifestations of early syphilis and of antisyphilitic treatment, the questioning being done by nonmedical personnel; (*b*) performance of serologic tests for syphilis on the blood of the men whose histories suggested previous syphilitic infection; (*c*) attempts to confirm previous antisyphilitic treatment by abstract; (*d*) questioning by a medical officer of the men whose histories suggested syphilis, and physical examination "when indicated," and (*e*) examination of the cerebrospinal fluid in all cases in which there was any doubt as to previous syphilitic infection. Of 2,451 soldiers subjected to this routine, 220 gave a history suggestive of syphilis or of antisyphilitic treatment. Of these, 55 men were found to be definitely syphilitic: 4 having early syphilis, 8 neurosyphilis and 43 latent syphilis. An additional 61 men were thought to have been cured by treatment. The authors stress the importance of an adequate history as compared with serologic testing and physical examination. None of the 51 men with latent syphilis or neurosyphilis showed physical evidence of syphilitic infection, and only 10 of these had doubtful or positive serologic reactions for syphilis.

(*b*) Contact Investigation: The finding of civilian contacts of soldiers seems somewhat less successful than the locating of contacts of a civilian clinic population. Norris, Doyle and Iskrant [29] present an analysis of reports on contacts in 4,641 cases of venereal disease submitted through the Third Service Command (comprising the states of Pennsylvania, Maryland and Virginia) Headquarters to local health departments from March 1 to December 31, 1942. A report of the disposition of 62.7 per cent of the cases was received from various health agencies located in thirty-seven states. Thirty per cent of the forms contained information sufficient for possible location of the contact; 27 per cent contained information sufficient for possible location of the contact or of the place of exposure, and 43 per cent contained wholly inadequate information. The most frequently reported contacts were pick-ups (64 per cent white, 45 per cent Negro) to whom no fee was paid; only 20 per cent were prostitutes. Encounter took place most frequently in taverns (35.6 per cent) and on the street (30.7 per cent), while the common site of exposure for white persons was the automobile and, for Negroes, the house or apartment. Of the 3,173 contacts reported to health authorities, only 22 per cent were located. Of these, approximately 50 per cent showed no definite evidence of infection, and of those found to be infected, 28 per cent were already under treatment at the time of investigation. Thus, among the 3,173 contacts only 234 previously unknown cases of disease were uncovered.

Unless more adequate information can be furnished by the Army to health departments and unless the latter can pursue contacts more diligently, this time-consuming and expensive method of case finding seems hardly justified, if the small yield obtained by these investigators is representative.

(*c*) Education: Stephenson and Mast [30] state that at least three points must be considered as to the need for and character of venereal disease education: the nature and extent of venereal disease, including its relative bearing on the main present day objective in the United States, a military victory; the conditioning effect of pub-

27. Doak, E. D.: The Venereal Disease Education Institute, J. Social Hyg. **30**:12 (Jan.) 1944.

28. Still, J. W., and Greenwald, E.: A Study of the Amount of Active Syphilis Found in a Group of Newly Inducted Soldiers, Ven. Dis. Inform. **25**:104 (April) 1944.

29. Norris, E. W.; Doyle, A. F., and Iskrant, A. P.: Venereal Disease Epidemiology, Third Service Command: An Analysis of 4,641 Contact Reports, Am. J. Pub. Health **33**:1065 (Sept.) 1943.

30. Stephenson, C. S., and Mast, G. W.: Venereal Disease Education in the U. S, Navy, J. Social Hyg. **30**:29 (Jan.) 1944.

lic attitudes and opinions, and the people specifically involved, their social and cultural backgrounds, and their present and future objectives in life.

The inescapable conclusion to be drawn from available data is that the venereal diseases are the most serious and dangerous preventable diseases to concern the medical officer of the Navy. Few problems of social organization have been so emotionally supercharged as those dealing with the control of venereal diseases. Around them, walls of taboo and morality have been created. Naturally, these factors influence both the policy of the Navy and the personal habits and opinions of the men in the Navy. By the very circumstance of his way of life, the Navy man presents a special problem. Any effort to influence his behavior while on liberty must be circuitous, indirect, unrecognized by him, made with tact and comprehension.

Venereal disease education is an important part of the responsibility of all naval medical officers, and in particular of the more than 50 venereal disease control officers now on duty. The Navy's program emphasizes the fact that one is dealing with diseases, not moral matters; that quack treatment is dangerous and ineffective, but Navy medical service is effective and reliable; that prostitutes are generally infected; that sex relations are unnecessary for preservation of health, and that prophylactic measures are effective if used properly. Fear as an educational motif has largely gone, but penalties linger on in the form of loss of pay and limitation of certain types of service and promotions. Further revision is in order. As educational mediums, in addition to the time-honored lecture, the Navy utilizes motion picture films and slides, pamphlets, posters and cartoons.

Anderson [31] states that the formulation of a program of education on venereal disease for the Army is based on certain fundamental tenets:

1. Continence is the most certain method of avoiding venereal disease.

2. The sex habits of the man of military age have been largely determined before he enters the Army.

3. Since there is a certain group who will expose themselves, prophylaxis must be given.

4. Instruction in prophylaxis must be given to all.

5. Instruction regarding venereal diseases and their prevention must be presented in a straightforward unemotional manner.

6. Instruction must appeal to all intellectual levels.

7. A wide variety of educational techniques must be employed in order to reach the largest number of individuals.

8. Whatever educational measures are used must appeal to the soldier.

9. Resort must be had to measures which frequently remind the individual of the basic instruction.

10. Instruction must not, however, be made monotonous.

11. Instruction must command the respect, not the ridicule, of the soldier.

12. Instruction of officers is essential to enlist their understanding and cooperation.

The author describes the educational measures undertaken by the Army in conformity with these tenets. The program is set forth in Training Circular 28, issued March 3, 1943.

(*d*) Venereal Disease Control: Turner [32] discusses the results achieved in the control of venereal disease in the United States Army since mobilization, the problems still requiring solution, and the manner in which wartime experience can contribute to the civilian program in peacetime. The achievements include, first, a decline in the venereal disease rate from 42.5 per thousand in 1940 to approximately 25 per thousand at present; second, a striking decrease in man days lost from duty, from 1,278 days for each 1,000 men per year to 368 days, and, third, the induction and treatment of over 55,000 civilians infected with venereal disease (including 20,000 syphilitic persons) between October 1942 and July 1943. These accomplishments are attributed to the recognition of venereal disease as primarily a medical problem, to the support of the program by those in authority and to the cooperation of civilian agencies. Continuing effort will be necessary for the solution of six immediate problems: (1) protection of troops in foreign stations, (2) reduction in rates for Negro troops, (3) research, particularly on prophylactic measures, therapeutic agents and epidemiologic data, (4) extension of educational efforts to the female civilian population, (5) maintenance of repression of prostitution and (6) the problem of the sexually delinquent teenage girl.

Regarding postwar civilian control programs, the author reemphasizes the value of prompt diagnosis and treatment, of contact investigation and of utilization of specially qualified medical personnel. It is suggested that education and community leadership can stimulate individual responsibility for good health, including freedom from venereal disease.

31. Anderson, G.: Venereal Disease Education in the Army, J. Social Hyg. **30**:20 (Jan.) 1944.

32. Turner, T. B.: Immediate Wartime Outlook and Indicated Post-War Conditions with Respect to the Control of the Venereal Diseases, Am. J. Pub. Health **35**:1309 (Nov.) 1943.

In another paper, Turner and Brumfield [33] discuss the annual rate of venereal disease in the United States Army from 1911 to the present. They point out that the present war has not been accompanied by the very high venereal disease rates observed in previous mobilizations.

A decline in the incidence both of syphilis and of gonorrhea has been noted. In 1940 the syphilis rate was 7.3 per thousand, whereas during the four month period beginning Nov. 1, 1942 the rate was under 5 per thousand on an annual basis. This is lower than any rate previously recorded in the Army.

The venereal disease rate in a command may be regarded as the product of three factors:

(1) the number of extramarital sexual exposures, (2) the number of exposures to infected persons, and (3) the proportion of exposures unprotected by prophylaxis. In considering syphilis, a fourth factor, namely, the number of susceptibles in the command, may be listed but as most of the soldiers have not been previously infected and hence are susceptible, this factor may be disregarded.

If the number of extramarital exposures can be reduced by education, by the repression of prostitution, and by the provision of substitutive activities, such as hard work, athletics, entertainment and other forms of recreation, the venereal disease rate will be affected proportionately. If the potential sources of infection can be reduced, fewer soldiers will become infected. If the soldier can be taught to use prophylaxis properly and provision made to have prophylactic materials readily accessible the venereal disease rate will thereby be reduced.

Outlining a brief history of venereal disease control in the United States Army, Pappas [34] summarizes some of the methods currently used to reduce the incidence of infections among men in military service. He recognizes as the keynote of venereal disease control the rigorous repression of prostitution, not losing sight of the role played by the nonprofessional, promiscuous "khaki-wacky" girl.

In the Army, great reliance is placed on the old Pershing dictum that "the prevention and control of venereal disease is the responsibility of the unit commander and for the personal performance of that duty he will be held strictly accountable."

(*e*) Treatment: Turner and Sternberg [35] report that in recent months the rate of venereal infection in the Army has been below preceding peacetime levels and less than half that recorded during the first World War. The number of days lost per thousand men annually has dropped from 1,278 in 1940 to a level of approximately 400 at the present time. In spite of this, patients infected with venereal diseases accounted for approximately 2,824,000 hospital bed days during the period from January 1942 to September 1943.

Determined efforts have been made to reduce this loss of manpower through measures aimed at the prevention of infection and through more efficient methods of treatment after infection. In prevention, reliance is placed on instruction of the individual soldier, on prophylaxis and, in cooperation with civilian agencies, on furthering those measures designed to reduce the potential sources of infection in the community.

The treatment of syphilis presents a real problem to the modern Army. Both the training program and the actual campaigns are so arduous that soldiers who are physically under par fall short of requirements and serve as a drag on the group to which they are attached. Although symptoms caused by early syphilis can be quickly eliminated, treatment itself may result in sickness, while treatment prolonged over long periods presents many administrative difficulties. Military requirements are best served by a system of treatment covering the shortest possible time consistent with safety to the patient and therapeutic efficacy.

On the recommendation of the Subcommittee on Venereal Diseases, National Research Council, the scheme of treating early and latent syphilis was substantially altered in July 1942. It was recommended that arsenoxide (mapharsen) be employed to the exclusion of other arsenicals. The scheme of treatment which had formerly been used, consisting of alternating courses of arsenical and bismuth preparations at weekly intervals for a period of one or two years, was replaced by a more intensive form of treatment. Twice weekly injections of arsenoxide, 60 mg. each, are administered for ten weeks. During the first five weeks concomitant weekly doses of 0.2 Gm. of bismuth subsalicylate (in oil) are given. For the following six weeks arsenoxide is omitted and one weekly dose of bismuth subsalicylate is given intramuscularly. Immediately arsenoxide is again given twice weekly for ten weeks, and during the last five weeks one concomitant dose of bismuth subsalicylate in oil is included. This treatment scheme covers a total period of twenty-six weeks. It is assumed that the end results of this system will not differ significantly from those of more prolonged methods and that "cures" will be effected in not less than 85 per cent of the cases of early syphilis. So far,

33. Turner, T. B., and Brumfield, W. A.: The Control of Venereal Disease in the Army, Am. J. Syph., Gonor. & Ven. Dis. **28**:133 (March) 1944.

34. Pappas, J. P.: The Venereal Disease Problem, United States Army, Mil. Surgeon **93**:172 (Aug.) 1943.

35. Turner, T. B., and Sternberg, T. H.: Management of the Venereal Diseases in the Army, J. A. M. A. **124**:133 (Jan. 15) 1944.

the short term results have been excellent. From the standpoint of toxic reactions and practicability of administration, the newer scheme appears to be definitely superior to the older one. Persons with primary or secondary syphilis are hospitalized for only a few days and treatment is carried on by unit medical officers.

Forms of intensive treatment, compressed within ten days or less, have not been adopted because of the risk of serious reactions and death inherent in these methods.

Because of fear of demotion or loss of pay, many members of the armed forces attempt to receive treatment for venereal diseases from civilian physicians. An editorial[36] states that civilian physicians are not aware of the fact that such men in the armed forces should be treated exclusively by commissioned Army and Navy officers. The following letter, received from the District Medical Officer of the Thirteenth Naval District, clarifies any misunderstanding so far as the Navy is concerned:

> This office has received inquiry from civilian physicians concerning the disposition of naval personnel who come to their offices seeking treatment for venereal diseases. Every effort should be made by Navy medical officers to locate and treat all venereally infected individuals under their jurisdiction. While the number of infections concealed in this manner may be relatively small, all personnel should be made cognizant of the fact that this form of concealment is subject to punishment.
>
> The information at hand indicates that most of the men seeking civilian treatment are in the higher rated groups of enlisted men. Civilian physicians should be indoctrinated, as a patriotic service, not to treat naval personnel for venereal diseases without informing Naval medical authorities. The surreptitious administration of sulfa drugs to naval personnel, particularly the aviation branch, may lead to fatal accidents.

Venereal Disease Control in Canada.—Williams[37] says that today, more than ever before, events are propitious for the final eradication of the venereal diseases. The immediate necessity of removing the threat of the venereal diseases to the health and efficiency of the armed forces is recognized by all. There are wholesome public interest, concern and support for measures directed against these diseases. This author states:

> The acquisition of venereal disease is comprised of two component parts, firstly, the source of infected individual and, secondly, the circumstances whereby the source is made accessible to the once healthy person. Based upon this dual concept of acquisition, control efforts logically consist of two steps—action dealing with the source, and action directed toward the removal of conditions rendering the source accessible. This orthodox approach has been largely neglected in the control of the venereal diseases. Interest and attention have been lacking because the epidemiology of syphilis and gonorrhea envisaged by a gun-barrel-vision perspective has seen only distantly and incompletely the problem of the source and has been entirely blind to the extensive surrounding visual field comprising those important factors related to the accessibility of the source. It is with the problem of accessibility of sources of syphilis and gonorrhea that this presentation is chiefly concerned. To this phase of the acquisition of the venereal diseases the term 'facilitation process' has been given.
>
> The facilitation process comprises those community conditions associated with the direct or indirect, witting or unwitting participation, usually for monetary gain, of third persons whereby individuals suffering from communicable venereal disease are made accessible for intimate exposure to healthy persons. The facilitation process centres chiefly around those community fornicatoriums known commonly as disorderly houses, and is associated also with certain beer parlours, hotels, dance halls, taxicab companies, rooming houses, apartment blocks, massage parlours, tourist camps, roadhouses and restaurants. The 'facilitators' or third-person participants are 'madams,' pimps and procurers, with whom are closely associated certain physicians, lawyers, finance companies and real-estate agencies. Less obvious but definitely in the ranks of the facilitators are the managers and owners of premises which facilitate healthy individuals toward sources of gonorrhea and syphilis. The attitude of certain civic administrations, health departments and law-enforcement agencies who condone community conditions which year after year facilitate large numbers of each new generation toward venereal infection and ill health, places the officials in these administrations in particular, and the public in general, in the position of being facilitators. Last but by no means least among the facilitators are those engaged in the commerce of alcohol for oral administration. Alcohol is the lubricant of the facilitation process. It enables the wheels of facilitation with smoothness and dispatch to hasten healthy citizens relentlessly to ill health and misfortune.

As to facilitation, much can be accomplished on a voluntary basis, and voluntary cooperation is always preferable to force and legal action. The sincere, cooperative facilitator, usually unwittingly involved, will immediately do everything in his power to assist. When an insincere facilitator is encountered, the seriousness of the continuing threat to the public health is such that the health department must discuss the matter with and send copies of the confirmatory correspondence to senior health officers, appropriate licensing boards, law-enforcement agencies and other civic administrative departments whose laws and by-laws are directed against the specific type of facilitation. Where cooperation and mutual understanding between the health department and facilitator fail, cancellation of the

36. Treatment of Venereally Infected Men in the Armed Forces, editorial, Northwest Med. **43**:130 (May) 1944.

37. Williams, D. H.: The Facilitation Process and Venereal Disease Control: A Study of Source Finding and Suppression of Facilitation in the Greater Vancouver Area, Canad. J. Pub. Health **34**:393 (Sept.) 1943.

licences of offending premises, such as rooming houses, massage parlors, hotels, beer parlors, taxicab companies and dance halls, stiff fines for landlords and real estate agencies, and lengthy jail sentences for the madam, the pimp and the procurer, have a salutary effect.

In an editorial [38] Williams states that the exigencies of war have forced the people of Canada to face the problem of venereal disease control. During the coming fiscal year, almost a million dollars will be spent by Canadian government health departments for this purpose.

Federal Government participation in the national effort has been three fold; a unified program of control for the Navy, Army, Air Force and Department of Pensions and National Health; the provision of grants-in-aid to Provinces amounting to $175,000; and the re-establishment of the Federal Division of Venereal Disease Control. The timely initial action on the part of the people of Canada and their governing agencies augurs well for the future health of the nation, provided the action is sustained and augmented.

A comprehensive approach to the problem has been announced to the Special Committee on Social Security of the House of Commons. There is a place for every citizen in the effort. A four-sector Canadian front against venereal disease is proposed. The individual components of this front are the health, welfare, legal and moral sectors. The ultimate object is to destroy syphilis and gonorrhea.

On the health sector a six-point strategy has been planned. This strategy envisages the application of basic principles used in overcoming other communicable diseases. The six points in brief are as follows:—

1. Wholesome, dignified health education concerning syphilis and gonorrhea.

2. Adequate diagnostic and treatment facilities for all persons suffering from venereal disease.

3. The suppression of quackery and charlatanry in the treatment of venereal disease.

4. Early, adequate, prenatal care, including blood tests for expectant mothers, to prevent the tragic innocent infection of babies.

5. General health examination, including blood tests for syphilis, on a voluntary basis, before marriage.

6. Effective measures to deal with unhealthy community conditions associated with the spreading of venereal dsease, and to deal wth persons who wilfully or unwittingly promote these conditions.

In another article, Williams [39] points out that the seriousness of the venereal disease situation in Canada has recently aroused the general interest of the public and their governing agencies. On July 1, 1943, a comprehensive control program was launched and a conference was held which was composed of members of the Canadian army, navy, air forces and health departments, as well as members of the Canadian, British and United States national research councils.

38. Williams, D. H.: Venereal Disease Control, editorial, Canad. M. A. J. **49**:210 (Sept.) 1943.

39. Williams, D. H.: Canada's First National Venereal Disease Control Conference, Am. J. Syph., Gonor. & Ven. Dis. **28**:286 (May) 1944.

Arsenoxide.—(a) Clorarsen and Phenarsine Hydrochloride. A comprehensive description of these drugs is given by the Council on Pharmacy and Chemistry.[40]

Since January 1936 the antisyphilitic agent, mapharsen, has been accepted by the Council on Pharmacy and Chemistry for inclusion in New and Nonofficial Remedies. Mapharsen (Parke, Davis & Co.) is a brand of 3-amino-4-hydroxyphenyl arsine oxide hydrochloride. . . .

In recent literature may be found reports of an arsenical antisyphilitic agent which apparently was discovered in the early part of this century but was cast aside as being too toxic for clinical use. Some years later there were published reports on its use in animals and in the treatment of yaws and human syphilis. It was not until 1941 that 3-amino-4-hydroxyphenyl dichloroarsine hydrochloride was found satisfactory for the treatment of syphilis; apparently the earlier studies were based on the use of an unbuffered compound which would provide a very low pH.

The preparations now available on the market contain sufficient alkaline buffering agent to make neutral a prepared solution for injection. They contain approximately 26 per cent of trivalent arsenic. At least four firms (Abbott Laboratories, Parke, Davis & Company, E. R. Squibb & Sons, Winthrop Chemical Company, Inc.) have been licensed to manufacture and make available for interstate commerce this substance, which is marketed at the moment as Phenarsine Hydrochloride (Winthrop) and Clorarsen (Squibb). The name proposed by the U. S. Pharmacopeia is Dichlorophenarsine Hydrochloride. One firm uses sodium citrate as a buffer, another sodium carbonate. On the addition of sterile distilled water to an ampul containing the mixture of dry dichlorophenarsine hydrochloride and alkaline buffer a reaction takes place with the result that arsenoxide is supposed to be formed. It has been claimed that the latter agent is the therapeutically active part of the compound.

In 1941 the therapeutic possibilities of buffered solution of dichlorophenarsine hydrochloride [clorarsen] were presented in a report (Tompsett, R. B., Downs, W. B., McDermott, Walsh, and Webster, Bruce: The Use of Clorarsen in the Treatment of Syphilis, J. Pharmacol. & Exper. Therap. **73**:412 [Dec.] 1941) based on a series of animal experiments and on a clinical study of 171 patients treated over a period of two years. . . .

The Council on Pharmacy and Chemistry has had under consideration for some time a brand of dichlorophenarsine hydrochloride. Consideration has not been completed pending the receipt of certain additional information, particularly that relating to stability. . . . At present, several interested persons claim that this agent is as stable or more stable than oxophenarsine hydrochloride (mapharsen), but the Council is withholding its final decision pending the receipt of more evidence. . . . The National Institute of Health has permitted a dating period of three years for 3-amino-4-hydroxyphenyl dichloroarsine hydrochloride (dichlorophenarsine hydrochloride), an indication that this body has procured evidence of stability which is satisfactory for a definite dating period. However, because the Subcommittee on Venereal Diseases of the Committee on

40. The Status of Dichlorophenarsine Hydrochloride: Trade Names Clorarsen, Phenarsine Hydrochloride, report of the Council on Pharmacy and Chemistry, J. A. M. A. **123**:208 (Sept. 25) 1943.

Medicine of the National Research Council and the Committee on Drugs and Medical Supplies concurred in a recommendation that the Council be requested to prepare a statement on the status of dichlorophenarsine hydrochloride and oxophenarsine hydrochloride (N. N. R. brand, mapharsen), the Council on the basis of information in the literature and that supplied by the National Research Council and one interested manufacturer adopted for immediate publication this general report. . . . This statement may have to be reversed as experience demands. At present, no brand of dichlorophenarsine hydrochloride stands accepted for inclusion in N. N. R., although the Winthrop Chemical Company has submitted its brand. Another brand which is available in interstate commerce, but which has not been submitted to the Council, is clorarsen made by E. R. Squibb & Sons.

During its consideration of these agents the Council questioned whether the medical profession will accept, generally, the names Dichlorophenarsine Hydrochloride and Oxophenarsine Hydrochloride, which have been proposed for inclusion in U. S. P. XII, first supplement, as official names for 3-amino-4-hydroxyphenyl dichloroarsine hydrochloride and 3-amino-4-hydroxyphenylarsine oxide hydrochloride respectively, phenarsine hydrochloride having been preempted as the name for a theoretical compound on which will be based new compounds. The Council is of the opinion that other nonproprietary designations might be chosen to advantage, names which would be less conducive to the coining of "tricky" names for sales promotion and which would be easier for the practicing physician to remember. Consideration might be given to applying the name "phenarsine" to the structural unit which has been designated "phenarsine hydrochloride" since the possibility is remote that salts other than the hydrochloride will ever be utilized in the preparation of such compounds. If this should be done, then the compound now designated "oxophenarsine hydrochloride" would become "phenarsine oxide," and the compound now designated "dichlorophenarsine hydrochloride" would become "phenarsine chloride." Or these compounds also might be called oxophenarsine and chlorophenarsine respectively. If Dichlorophenarsine Hydrochloride and Oxophenarsine Hydrochloride are retained as U. S. P. names, it appears not unlikely that the label will stress Dichlorophenarsine and Oxophenarsine, with Hydrochloride appearing in smaller letters. Such a procedure, it is understood by the Council, would be acceptable to the National Institute of Health and might offer some aid to the physician.

The point of this communication by the Council on Pharmacy and Chemistry was intended to be a clarification of the confusion in the minds of practicing physicians between three arsenical compounds now available on the open market. These are mapharsen (Park, Davis & Company), clorarsen (E. R. Squibb & Sons) and phenarsine hydrochloride (Winthrope Chemical Company). For all practical purposes these three drugs on solution and on intravenous injection are identical and may be employed interchangeably with the necessary minor alterations for dosage suggested by the manufacturers.

Further experiences with phenarsine hydrochloride (dichlorophenarsine hydrochloride) are recorded by Boardman and Kaldeck,[41] who have given 1,299 injections to 112 patients with various stages of syphilis. Therapeutic efficacy as determined by disappearance of early lesions and reversal of serologic reactions was satisfactory. Minor reactions, chiefly gastrointestinal, were relatively frequent, but in only 7 cases was it necessary to discontinue the use of the drug. Severe gastrointestinal reactions occurred in 5; dermatitis and jaundice in 1 each. As administered intravenously, this drug is essentially identical with mapharsen.

Few data have been published on the blood levels, distribution in the tissues and excretion of arsenoxide. Those articles which have appeared have dealt primarily with intensive treatment. Henning and Kampmeier [42] present studies on blood levels of arsenic and its excretion after single injections of clorarsen (3-amino-4-hydroxyphenyldichloroarsine hydrochloride). Determinations of arsenic were made by the colorimetric method of Chaney and Magnuson. For the determination of blood levels of arsenic after one injection of clorarsen, arsenoxide and neoarsphenamine, samples were drawn at varying intervals after the injection, usually from five to fifteen minutes. Determinations were usually made on whole blood, although in some instances the blood plasma was studied. The cerebrospinal fluid of 2 patients treated with clorarsen was examined. The excretion of arsenic after a single therapeutic injection of clorarsen and mapharsen was followed for six days after the injection. Urine and stools were examined for arsenic during a two day control period prior to treatment and during the days following treatment.

In 6 instances after an injection of 11.4 or 17 mg. of arsenic in the form of clorarsen, determinations being made from five to sixteen minutes after the injection, 0.05 mg. of arsenic or less per hundred cubic centimeters could be found in the blood. Results were identical with arsenoxide and with clorarsen. Neoarsphenamine in doses providing comparable amounts of arsenic produced much higher levels of arsenic in the blood, 0.1 mg. per hundred cubic centimeters. The patients who received clorarsen excreted 18.6 to 20.8 per cent of the arsenic in the urine within six days; those receiving mapharsen, 22.6 per cent. From 21.6 to 43.4 per cent of the in-

41. Boardman, W. P., and Kaldeck, R.: Phenarsine Hydrochloride in the Treatment of Syphilis, New England J. Med. **230**:12 (Jan. 6) 1944.

42. Henning, H. B., and Kampmeier, R. H.: Blood Level and Excretion of Arsenic Following Single Injections of Clorarsen, Arch. Dermat. & Syph. **48**:297 (Sept.) 1943.

jected arsenic was excreted in the stools of the patients receiving clorarsen during the six days following the administration of the drug. The patients who received clorarsen excreted from 41.1 to 62.1 per cent of the arsenic in urine and feces within a period of six days.

The authors conclude that clorarsen and mapharsen are removed from the blood stream so rapidly that practically no arsenic can be demonstrated after five to fifteen minutes, whereas traces of neoarsphenamine may remain in the blood for as long as twenty-four hours.

(*b*) Mapharsen: Brown, Kolmer and Rule [43] have determined the toxicity and therapeutic effectiveness of mapharsen administered orally. When the drug was given by this route, the maximum tolerated single dose was more than 0.5 Gm. per kilogram of body weight, this being at least three times less than the intramuscular dose and at least thirty-three times less than the intravenous doses. The data provided as to the therapeutic effectiveness of mapharsen after oral administration may be misleading because the criterion of "cure" was lymph node transfer at the early date of eight weeks following treatment.

From estimations of the urinary excretion of arsenic it is apparent that mapharsen is readily absorbed from the gastrointestinal tract. Apparently arsenic is absorbed and excreted at about the same rate after the oral administration of mapharsen, arsphenamine and neoarsphenamine. Arsenious oxide is absorbed more rapidly but is ineffective therapeutically on both intravenous and oral administration.

Phenyl Arsenoxides.—Ehrlich's original hypothesis that chemotherapeutic agents can exert their therapeutic effect only if bound by the parasite and that their toxic action is due to a similar combination with vital tissues of the host is upheld in respect to the arsenical drugs by a study reported by Hogan and Eagle.[44] They summarize their evidence as follows:

1. In a series of phenyl arsenoxides varying twenty-fold in toxicity, the amount of each (acid-substituted compounds excepted) bound by red blood cells in vitro was in proportion to its systemic toxicity.

2. A similar variation was found in the amount of arsenical bound by circulating red blood cells immediately after intravenous injection. The non-toxic compounds were not bound to the same degree as toxic compounds, and left the blood stream at a faster rate.

3. The amount of arsenic remaining in the liver and kidney 24 or 48 hours after the intravenous injection of arsenoxides or arsenic acids was proportional to their toxicity.

4. The rate of excretion of phenyl arsenoxides (acid-substituted compounds excepted) was also a function of their toxicity. The non-toxic compounds, not bound by body cells, were excreted rapidly; while the toxic compounds were excreted slowly, in inverse proportion to their toxicity.

5. At dosages which produced equivalent toxic effects (the LD_{50} level), tryparsamide, phenyl arsonic acid and phenyl arsenoxide resulted in comparable tissue levels, despite a 500-fold difference in absolute arsenic dosage.

6. It is therefore suggested that the varying systemic toxicity of arsenicals is primarily determined by the varying degree to which they are bound by, and thus block, essential functional groups in vital organs. The chemical nature of these groups is discussed in the text.

7. Acid-substituted phenyl arsenoxides are only an apparent exception to this generalization. Although fairly toxic, they were bound to only a minimal degree by red blood cells in vitro or in vivo. After intravenous injection, they were at first excreted rapidly, as much as 40 per cent appearing in the urine in one hour. The excretion was, however, abruptly curtailed after approximately four hours; and death in white mice injected at the LD_{50} level was characteristically delayed as compared with death resulting from other phenyl arsenoxides. It seems probable that most acid-substituted phenyl arsenoxides are not toxic as such, consistent with their lack of affinity for red blood cells, and their initially rapid excretion. The sudden curtailment of urinary excretion, and the delayed death of mice, suggest that they are converted by the body to other compounds which can combine with vital chemical groupings in the tissues, and which are toxic by virtue of that combination.

Bismuth Sodium Paraaminophenylarsonate.—In the past, numerous attempts have been made to combine trivalent or pentavalent arsenicals with bismuth. The resulting compounds have never been particularly satisfactory chemotherapeutic agents for syphilis.

Bruce and his co-workers [45] report on the toxicity of bismuth sodium paraaminophenylarsonate for experimental animals and their tolerance of it. This substance is described as a combination of sodium paraaminophenylarsonate and bismuth subgallate. It is a stable water-soluble product. Each 2 cc. of solution contains 21 to 22 mg. of bismuth and 7.8 mg. of pentavalent arsenic as bismuth sodium paraaminophenylarsonate and 7.8 mg. of arsenic as sodium cacodylate. It is marketed as arseno-bismulak and is intended for intramuscular use in the treatment of syphilis. For the determination of intravenous toxicity, 198 rats and 18 rabbits were employed. For the determination of intramuscular toxicity, 132 rats, 38 rabbits, and 15 dogs were utilized. The drug appears to be well tolerated. Practically the only

43. Brown, H.; Kolmer, J. A., and Rule, A. M.: The Oral Administration of Mapharsen in the Treatment of Experimental Syphilis in Rabbits, Am. J. Syph., Gonor. & Ven. Dis. **27**:480 (July) 1943.

44. Hogan, R. B., and Eagle, H.: The Pharmacologic Basis for the Widely Varying Toxicity of Arsenicals, J. Pharmacol. & Exper. Therap. **80**:93 (Jan.) 1944.

45. Bruce, F.; Chase, H. F.; Lehman, A. J., and Yonkman, F. F.: Toxicity and Tolerance of Bismuth Sodium Para-Aminophenylarsonate (Arseno-Bismulak), Urol. & Cutan. Rev. **48**:183 (April) 1944.

visible evidence of tissue injury was found in the kidneys, and that only after the administration of doses approaching lethal amounts.

Lehman, Chase and Bruce [46] studied in white rats and rabbits the toxicity and therapeutic efficacy of a water-soluble organic combination of bismuth subgallate and sodium arsanilate designated as bismuth sodium paraaminophenylarsonate. Intramuscular administration of a total of 0.478 cc. of solution (3.72 mg. of arsenic and 5 mg. of bismuth) per kilogram of body weight was found to cure rabbit syphilis, as determined by lymph node transfer. One third of the total dose was administered every other day for three doses. The 50 per cent mortality dose for rabbits, with the same method of administration, was found to be 1.75 cc. (13.65 mg. of arsenic and 18.37 mg. of bismuth) per kilogram of body weight. Adopting the most conservative figures, the authors estimate the therapeutic index as 1:3.

Detoxication of Arsenicals.—Further discussion of the detoxifying action of ascorbic acid against neoarsphenamine is presented by McChesney, Barlow and Klinck.[47] In preliminary experiments a number of organic acids were studied, including ascorbic, isoascorbic, d-glucoascorbic, lactic, pyruvic, succinic, malic, mandelic, aspartic, gluconic and 2-ketogulonic acids and l-cysteine. Of these, only ascorbic, isoascorbic, d-glucoascorbic and lactic acid showed evidence of a detoxifying action. In addition to the above, paraaminobenzoic acid was found to be about equally effective as a detoxicant against neoarsphenamine.

The most beneficial effect was obtained when the arsenical and the protective agent were injected intravenously in the same solution. A favorable effect, though somewhat lessened, was obtained when the solutions were injected simultaneously at different sites. The authors agree that the function of the ascorbic acid appears to be primarily that of preventing oxidation and that the mechanism of action of p-aminobenzoic acid is different. Experimental studies on rabbits infected with the Nichols strain of T. pallidum indicate that the addition of ascorbic or isoascorbic acid to one arsenical compound did not alter its therapeutic efficacy.

Sandground,[48] continuing studies previously reported, states:

> It is here shown that many aromatic compounds have the property of inhibiting the lethal consequence of massive doses (ca. L. D. 90+) of pentavalent aromatic arsenical in rats. In addition to the three isomeric forms of aminobenzoic acid, a high order of protection is conferred by the hydroxy- and nitro-analogues of PABA, as well as by other substituted compounds which are readily soluble and not in themselves highly toxic to the test animal. Inasmuch as benzoic, phenyl acetic, and phenyl propionic acids are all highly effective, it appears that a high degree of structural similarity between detoxicant and toxicant is not an essential of the mechanism underlying the detoxication phenomenon.

Bismuth.—The long known intolerance to and therapeutic inefficacy of the intravenous use of bismuth compounds has again been confirmed. Hanzlik and Ludena [49] found that when sobisminol solution was injected slowly by intravenous drip it was more toxic for syphilitic than for normal rabbits. Because the drug was so toxic, an adequate dosage could not be given to heal syphilitic lesions of rabbits. Ascorbic acid did not reduce the toxicity of the drug. The cause of the lowered tolerance to sobisminol solution when given intravenously to syphilitic rabbits is not known.

A new bismuth compound, dihydroxypropyl bismuthate, has been introduced for the oral treatment of syphilis by Nomland and others.[50] This drug, when given orally in the recommended dosage of 100 mg. three times daily, is rapidly and fairly uniformly absorbed, producing blood concentrations of bismuth which are somewhat higher than those obtained by intramuscular injection of bismuth subsalicylate. A high concentration of bismuth was also found in the spinal fluid. The drug was given clinical trial (150 to 300 mg. per day) in 5 patients with early syphilis and in 3 with benign late lesions. Moist genital lesions remained darkfield positive for ten, twenty and twenty-one days after the institution of treatment. The lesions of 2 patients with late cutaneous syphilis healed in one week and in sixty days respectively. The authors believe that this response of lesions, both early and late, approximates that which is observed follow-

46. Lehman, A. G.; Chase, H. F., and Bruce, F.: Bismuth Sodium Para-Aminophenylarsonate: A Preliminary Summarizing Report of Its Pharmacologic Actions and Therapeutic Value in Experimental Syphilis, Urol. & Cutan. Rev. **47**:575 (Oct.) 1943.

47. McChesney, E. W.; Barlow, O. W., and Klinck, G. H.: The Detoxication of Neoarsphenamine by Means of Various Organic Acids, J. Pharmacol. & Exper. Therap. **80**:81 (Jan.) 1944.

48. Sandground, J. H.: Studies on the Detoxication of Organic Arsenical Compounds: V. Additional Detoxicants for Pentavalent Arsenicals, J. Pharmacol. & Exper. Therap. **80**:393 (April) 1944.

49. Hanzlik, P. J., and Ludena, F. P.: Intravenous Administration of Sobisminol Solution: Toxicity in Normal and in Syphilitic Rabbits, Arch. Dermat. & Syph. **48**:35 (July) 1943.

50. Nomland, R.; Wheeler, L. M.; Carney, R. G.; Kuever, F. A., and Gross, E. G.: Dihydroxypropyl Bismuthate Orally in the Treatment of Syphilis: A Clinical and Chemical Study (Preliminary Report), Am. J. Syph., Gonor. & Ven. Dis. **28**:68 (Jan.) 1944.

ing the intramuscular administration of bismuth. In 1 of 19 patients treated with this drug, a widespread eczematoid dermatitis developed. No other toxic reactions were observed; albuminuria did not occur.

Since bismuth and lead belong to the same series in the periodic system of the elements, with a striking similarity in their deposition in the skeletal system, and because acidosis tends to promote the mobilization and excretion of lead, Brown, Kolmer and Rule [51] have studied the effect of acidosis induced by the administration of ammonium chloride on the mobilization and excretion of bismuth. The daily administration of 0.1 Gm. of ammonium chloride per kilogram to normal and to syphilitic rabbits was found to increase the mobilization and urinary excretion of elemental bismuth after intramuscular injections of bismuth subsalicylate. The increased mobilization of bismuth following ammonium chloride acidosis did not appear to increase the effectiveness of bismuth in the treatment of acute syphilitic orchitis in rabbits.

Translating their findings to the treatment of human syphilis, the authors believe there is no reason to administer ammonium chloride for the purpose of mobilizing bismuth from storage depots. The presence of bismuth in these depots apparently is without untoward effects; its mobilization appears not to increase the therapeutic effect significantly. Moreover, since acidosis increases the toxic effects of the drug, it is inadvisable to give ammonium chloride to bismuth-treated patients predisposed to acidosis.

Potassium Iodide.—Because of gastric irritation and of variation in dosage when potassium iodide is prescribed in liquid form, Garfield [52] suggests the use of enteric-coated pills containing 1 Gm. each. None of 12 syphilitic patients treated with such pills had gastric disturbances, although 3 did show an idiosyncrasy to the drug. As judged by the healing of lesions, chiefly gummas, absorption seemed to be adequate with this method of administration.

PENICILLIN

Toxicity of Penicillin.—Hamre and her associates [53] have studied the toxicity of the drug in experimental animals and discuss their findings as follows:

> In the literature, reports of experiments in which animals have been given a single intravenous dose of penicillin have shown that its acute toxicity is very low. Since penicillin is not a pure substance and preparations vary in potency, the exact toxic dose has not been established. Obviously the assay of toxicity should be based on Florey units as well as milligrams of substance given. For white mice, we found the lethal intravenous dose to be about 90,000 F. U. (1 gm.) per kg. using a preparation containing 90 F. U. per mgm., but in another experiment using more highly purified material (50 F. U. per mgm.) 250,000 F. U. per kg. caused only a slight reaction. . . . These results indicate that purification removes some of the substances responsible for this acute toxicity. Very little data have been given on toxicity for other animals. In our experiments 111,000 (1.2 gm.) per kg. was a lethal dose for rabbits and 75,000 F. U. (0.8 gm.) per kg. killed guinea pigs when given intravenously. . . .
>
> When penicillin was given subcutaneously over a period of several days or weeks to mice, rabbits and guinea pigs, the picture was altered. All animals showed a severe reaction at the site of injection. Edema of the subcutaneous tissue, infiltration of monocytes and polymorphonuclear leukocytes, and destruction of subjacent voluntary muscle were found in all animals, and in the rabbits and 3 of the guinea pigs, there were hemorrhages. Other than this, the mice and rabbits tolerated the penicillin injections well. . . . In the guinea pigs, however, prolonged subcutaneous injections of penicillin prepared at different places and by different methods caused death.
>
> Until penicillin is prepared in a chemically pure state, it will not be possible to determine whether or not contaminating substances are responsible for all of the toxicity of penicillin preparations. None of the preparations tested in these experiments were non-toxic, even a preparation having a 10-fold increase in purity was still toxic although it failed to kill all of the guinea pigs injected. It is possible that further purification will remove all of the toxic substances. However, it should be stressed that the penicillin preparations used were all as pure as, and in many cases purer than those at present in use in human treatment; and some were actually used in such treatment.
>
> The fact that present preparations are toxic for guinea pigs when given subcutaneously does not mean that penicillin is toxic for man. When treated with the same dose of penicillin per kg. as that given to man, guinea pigs did not die and, in fact, failed to show any signs of toxicity. However, it is suggested that chronic toxicity for man be borne in mind and that care be taken to administer penicillin in several widely separated places when given subcutaneously or intramuscularly.

Penicillin for Spirochetal Infection.—As will presently appear, penicillin appears to offer great promise in the treatment of syphilis. For this reason, this review devotes considerable space to the available literature on the experimental use of this drug for spirochetal infections other than syphilis, because of the possible implications as to syphilis. The studies so far available on syphilis itself, both experimental and clinical, are likewise reviewed, on the basis not only of published papers but of certain communi-

51. Brown, H.; Kolmer, J. A., and Rule, A. M.: The Influence of Ammonium Chloride on the Mobilization and Excretion of Bismuth, Am. J. Syph., Gonor. & Ven. Dis. **27**:501 (July) 1943.

52. Garfield, W. T.: A New Method of Giving Potassium Iodide, New England J. Med. **229**:971 (Dec. 23) 1943.

53. Hamre, D. M.; Rake, G.; McKee, C. M., and MacPhillamy, H. B.: The Toxicity of Penicillin as Prepared for Clinical Use, Am. J. M. Sc. **206**:642 (Nov.) 1943.

cations which have recently been presented before various medical societies and even in advance of their actual publication. This procedure is adopted because of the widespread interest attaching to the subject, the release of penicillin for the treatment of syphilis in civilian practice by the Office of Civilian Penicillin Distribution of the War Production Board, and the consequent desirability of rapid dissemination of information.

Penicillin for Spirochetal Infections other than Syphilis.—Lourie and Collier [54] have studied the effect of penicillin on certain spirochetal and protozoal infections in experimental animals. With infections caused by Spirochaeta recurrentis in mice, a single intravenous dose of 500 units of penicillin given fifteen minutes after inoculation failed to prevent an infection of the blood stream. Three such doses given at hourly intervals protected 1 of 5 mice, but five doses of 500 units on two successive days protected each of 2 mice so treated. In already established infections, the blood was usually cleared of parasites within twenty-four hours by 250 units given subcutaneously whether in a single injection or in 5 fractional doses of 50 units each at hourly intervals; most animals so treated had a relapse within a month. In Spirillum minus infectious in mice, a single intravenous dose of 250 units given fifteen minutes after inoculation was protective. In established infections, temporary sterilization of the blood was accomplished with as little as 10 units given subcutaneously; but the single curative dose was at least 1,000 units. With repeated doses (ten in two days), the curative range was 100 to 500 units. Penicillin had no action against infections in mice caused by Trypanosoma rhodesiense, Trypanosoma congolense or Trypanosoma cruzi and was likewise ineffective in avian malaria (Plasmodium relictum).

After numerous experiments to determine the virulence of their strain of Leptospira and the toxicity of penicillin in guinea-pigs, Heilman and Herrell [55] carried out two experiments:

In the first of these, 14 guinea pigs infected with leptospirosis were treated with 3,000 to 5,000 units of penicillin (salt and vehicle of solution or suspension not named) daily, given in 5 injections per day for from five to seven days. Protocols are not supplied, so that it is not possible exactly to determine dosage, but from the data described it appears that the total dose ranged roughly between 50,000 and 115,000 units per kilogram of body weight. Of these 14 treated animals, none appeared to acquire leptospirosis, but 7 died of what was considered to be the toxic effects of penicillin. Of 14 untreated infected controls, 13 contracted leptospirosis. In a second experiment, 32 infected guinea pigs were treated with subcutaneous injections of calcium penicillin suspended in sesame oil, 800 units daily, in three divided doses, for seven days. The weight of the animals is stated to be "approximately 200 Gm."; hence the total dose of penicillin was about 28,000 units per kilogram of body weight. Treatment was commenced seventeen to twenty-four hours after inoculation. None of the 32 treated animals died of leptospirosis. Three of the group did die, but death was attributed to the toxic effect of penicillin. Of the untreated animals, 29 died of leptospirosis, a mortality rate of 91 per cent. The authors conclude that the effectiveness of penicillin against infection produced by Leptospira icterohaemorrhagiae in guinea pigs, as described in their experiment, makes it seem reasonable to suspect that penicillin will be useful in the treatment of Weil's disease and other leptospiral infections in man. No attempt is made, however, to define the probable minimum effective dose.

The clinical syndrome known as rat bite fever is produced in man by two apparently different pathogenic organisms, Spirillum minus and Streptobacillus moniliformis. Heilman and Herrell [56] have studied the effect of penicillin in mice infected with these organisms. Regardless of the body weight of the animals (which in the several experiments ranged from 14 to 26 Gm.) a standard dose of penicillin of 1,000 units per day, in five divided doses, was employed over periods ranging from five and one-half to seven days. The range of total dosage in terms of units per kilogram of body weight is not given; but as nearly as it can be determined from the inadequate protocols, this range was from about 266,000 to 437,000 units. With these enormous doses, corresponding to 15,000,000 to 26,000,000 units for a 60 Kg. man, the drug was found to be effective in 25 mice infected with Spirillum minus and 43 mice infected with Streptobacillus moniliformis.

54. Lourie, E. M., and Collier, H. O. J.: The Therapeutic Action of Penicillin on Spirochaeta Recurrentis and Spirillum Minus in Mice, Ann. Trop. Med. **37**:200 (Dec.) 1943.

55. Heilman, F. R., and Herrell, W. E.: Penicillin in the Treatment of Experimental Leptospirosis Icterohaemorrhagica (Weil's Disease), Proc. Staff Meet., Mayo Clin. **19**:89 (Feb. 23) 1944.

56. Heilman, F. R., and Herrell, W. E.: Penicillin in the Treatment of Experimental Infections with Spirillum Muris and Streptobacillus Moniliformis (Rat-Bite Fever), Proc. Staff Meet., Mayo Clin. **19**:257 (May 17) 1944.

Heilman and Herrell [57] treated 22 mice infected with relapsing fever (Borrelia novyi) with a standard total dose of 4,000 units of penicillin administered in divided doses over a four day period. In units per kilogram of body weight (not supplied by the authors) this represents about 200,000 units per kilogram, corresponding to a total dose of 12,000,000 units for a 60 Kg. man. All the treated animals appeared to be cured, whereas 75 per cent of 28 untreated controls died. The authors say:

> In this study the dose of penicillin used was relatively large. It seems probable that smaller doses . . . would be effective in the treatment of experimental relapsing fever in mice, particularly if the inoculum was less virulent and if the drug could be administered at short intervals throughout the night as well as during the day. . . . It is hoped that as a result of these studies a more effective agent for the treatment of relapsing fever will be made possible in the form of penicillin.

An even more sketchy and inadequate report appears from Augustine, Weinman and McAllister.[58] These workers report the results of the administration of a total of 9,000 units of penicillin intraperitoneally, in divided doses given every three hours for forty-eight hours, to six (sic) mice! The dose in units per kilogram of body weight is not supplied, but it apparently corresponds to a total dose of about 22,000,000 units to a man.

Parenthetically, in the several experiments just described, Heilman and Herrell appear to have been the first to employ penicillin in suspension in oil rather than in aqueous solution, in the effort to provide delayed absorption and excretion. They present no data to indicate, however, the extent to which this aim was accomplished. The suggestion that the drug be used in this manner is an important one in the therapy of syphilis, which requires much further exploration. The frequent injections apparently necessary with aqueous solutions require hospitalization, an impractical procedure for large numbers of syphilitic patients in civilian practice.

These several studies of Lourie and Collier, Augustine and his associates, and Heilman and Herrell may be regarded as of only qualitative, not quantitative, importance. While they indicate that penicillin, in the enormous doses employed, is effective against experimental relapsing fever, leptospirosis and rat bite fever, the data are too incomplete to afford any indication of the probable effective therapeutic dose for man. The experiments described require repetition and much amplification.

The deficiencies in these purely qualitative studies have been partially remedied by a careful quantitative study (Eagle and Magnusen [59]) of the therapeutic efficacy of penicillin in relapsing fever infections in mice and rats. These workers investigated the effect of graduated doses of penicillin in 66 infected rats and 32 infected mice. The drug was given intraperitoneally every 4 hours (at 8 a. m., noon, 4 and 8 p. m. and midnight) on each of two successive days. For rats, the range of total dose employed was from 10,000 to 640,000 units per kilogram, for mice, from 20,000 to 640,000 units per kilogram. The authors report:

> The total dosages of penicillin which "cured" 50 per cent of white rats and mice infected with *Borrelia novyi* were 130,000 and 100,000 units per kilogram respectively. Approximately 400,000 units per kilogram were necessary to cure more than 95 per cent of the animals. . . . If these results can be translated to man, they imply that the curative dose of penicillin in man would be on the order of 25,000,000 units. Unless relapsing fever is more amenable to treatment in man than it is in these experimental animals, or unless other strains of the organism prove more susceptible to penicillin, the therapeutic use of the drug would not appear warranted except in arsenic-resistant cases, at least until such time as it is available in larger quantities.

Penicillin for Experimental Syphilis.—In a paper presented before the Society of American Bacteriologists in May 1944, only a brief abstract of which has so far appeared in print, Eagle and Musselman [60] discuss the spirocheticidal action of penicillin in vitro. With the so-called Reiter strain of T. pallidum (nonpathogenic), it was shown that:

1. In those mixtures containing small numbers of organisms, all the spirochetes were killed by as little as 0.062 Oxford unit per cubic centimeter (approximately a 1 : 25,000,000 concentration of the drug). As the number of organisms increased, progressively more penicillin was required to effect complete sterilization. These data were, however, related to the deterioration of penicillin under the conditions of the experiment.

57. Heilman, F. R., and Herrell, W. E.: Penicillin in the Treatment of Experimental Relapsing Fever, Proc. Staff Meet., Mayo Clin. **18**:457 (Dec. 1) 1943.

58. Augustine, D. L.; Weinman, D., and McAllister, J.: Rapid and Sterilizing Effect of Penicillin Sodium in Experimental Relapsing Fever Infections and Its Ineffectiveness in the Treatment of Trypanosomiasis (Trypanosoma Lewisi) and Toxoplasmosis, Science **99**: 19 (Jan. 7) 1944.

59. Eagle, H., and Magnusen, H. J.: The Therapeutic Efficacy of Penicillin in Experimental Relapsing Fever, J. Bact. **47**:21 (May) 1944. Eagle, H.; Magnusen, H. J., and Musselman, A. D.: The Therapeutic Efficacy of Penicillin in Relapsing Fever Infections in Mice and Rats, Pub. Health Rep. **59**:583 (May 5) 1944.

60. Eagle, H., and Musselman, A. D.: The Spirocheticidal Action of Penicillin in Vitro (Treponema Pallidum—Reiter Strain), J. Bact. **47**:22 (May) 1944.

2. T. pallidum was killed by penicillin, as judged by its ability to grow out in subculture, many hours before its motility was lost.

The authors say:

Penicillin was found to be equally active against the Nichols, Noguchi and Kazan strains, also purporting to be cultures of *S. pallida*, and a culture of mouth spirochetes. . . .

The degree to which results obtained with cultured organisms may be carried over to pathogenic *S. pallida* is an open question. . . . Suspensions from rabbit testicular chancres were not immobilized in 2 to 6 hours by concentrations of penicillin up to and including 500 units per cc. However, since the motility of the highly susceptible cultured spirochetes were also unaffected in that time period and in those concentrations, this observation does not exclude a direct spirocheticidal action. . . .

. . . A single injection in man of e. g., 30,000 units would, if uniformly distributed through the tissues, result in a temporary tissue fluid level on the order of 1 unit per cc. This is so many times the concentration here found to be effectively spirocheticidal in vitro (0.01 to 0.05 units per cc.) that even the rapid excretion of this compound would nevertheless be consistent with the maintenance of an effective concentration for a period of hours.

Dunham and his coworkers,[61] employing Eagle's in vitro technic, found penicillin to immobilize spirochetes (Nichols strain obtained in the supernate after centrifugation of rabbit testicular chancre emulsion) in the relatively high concentration of 800 to 1,600 Oxford units per 0.8 cc. of other antibiotics tested. Gliotoxin had a marked immobilizing action; aspergillic acid, bromoaspergillic acid and fumigacin were less effective. An observation recorded by these authors suggests the possibility of penicillin-resistant strains of T. pallidum. Spirochetes recovered from one rabbit treated with subcurative doses of penicillin proved to be relatively resistant to the drug by the in vitro test.

Dunham, Hamre and Rake[62] have been able to produce a penicillin-resistant strain of T. pallidum. An abstract of their report reads as follows:

Intratesticular inoculations in rabbits were made with suspensions of the popliteal lymph nodes of rabbits which had been infected intracutaneously with *T. pallidum* and then treated with varying amounts of penicillin. From two of the rabbits which had developed skin chancres, spirochetes were isolated by this means. One of these rabbits had developed a chancre similar in all respects to those of the untreated controls, while the chancre of the other developed 3 to 4 weeks later and was abortive. After one intratesticular passage, *in vitro* tests were performed to determine whether any change had occurred in the resistance of the spirochetes to penicillin.

The spirochetes derived from the rabbit with the large chancre showed no difference from the parent strain in their susceptibility to penicillin. Those derived from the rabbit with the abortive lesion were distinctly more resistant to the action of penicillin than the parent strain. This characteristic has persisted after further intratesticular passages.

Sufficient treatment with penicillin to modify the course of an experimental syphilitic infection, but not enough to cure the animal, resulted in the development of a penicillin-fast strain. This emphasizes the necessity for adequate treatment with penicillin in clinical cases.

Penicillin in the Treatment of Syphilis in Human Beings.—Preliminary reports by Mahoney, Arnold and Harris[63] record observations made on 4 patients with primary syphilis who were treated with penicillin only. A study of the usefulness of the drug in the management of syphilis was undertaken after limited experimentation with animals indicated that penicillin possessed some spirocheticidal activity. The early results in experimental animals indicate that the time-dose relationship will prove to be as important in this therapy as in the use of other chemotherapeutic agents. Failures to sterilize experimentally infected animals with treatment schedules which utilize minimal amounts of the drug over a brief treatment period are predictable. The results from treatment schedules which utilize larger amounts of the drug and more prolonged treatment periods will require observation for approximately one year for complete evaluation.

The first 4 patients to be treated with this drug have now been observed for a period sufficiently long to permit comparison with results produced by more conventional forms of treatment. In each case, there was a single penile lesion which showed spirochetes on dark field examination. The duration of the ulcerations before the start of treatment was approximately eight days. No systemic treatment other than that with penicillin was employed in any case. Treatment with penicillin consisted of an intramuscular injection of 25,000 units of the drug at four hour intervals night and day for eight days. The total number of injections was forty-eight, and the total amount of the drug was 1,200,000 units. The drug was injected in the gluteal muscles. Dark field studies were

61. Dunham, W. B.; Hamre, D. M.; McKee, C. M., and Rake, G.: Action of Penicillin and Other Biotics on Treponema Pallidum, Proc. Soc. Exper. Biol. & Med. **55**:158 (March) 1944.

62. Dunham, W. B.; Hamre, D. M., and Rake, G.: The Development of a Penicillin-Fast Strain of Treponema Pallidum, J. Bact. **47**:22 (May) 1944.

63. Mahoney, J. F.; Arnold, K. C., and Harris, A.: Penicillin Treatment of Early Syphilis: A Preliminary Report, Am. J. Pub. Health **33**:1387 (Dec.) 1943; Penicillin Treatment of Early Syphilis: A Preliminary Report, Ven. Dis. Inform. **24**:355 (Dec.) 1943.

carried out at four hour intervals following the beginning of treatment, and no spirochetes could be found in any case after the sixteenth hour. During the first eight hours of treatment, the patients complained of general malaise and mild headache. There were elevations of temperature not in excess of 2 degrees F. The penile lesion became painful, and the regional lymph nodes became enlarged and tender. One patient displayed a maculopapular eruption resembling secondary syphilis over the trunk and thighs. The eruption was of short duration. There were no symptoms which could be attributed to a toxic response to the drug.

All patients were carefully followed with several complement fixation and flocculation technics. The results of these serologic studies indicated that the therapy was responsible for more or less rapid and complete disappearance from the blood stream of the reacting substance measured by the various tests and usually associated with activity in early syphilis. All the patients' serologic reactions became negative. Further observation on this group of patients will be maintained on a weekly basis for as long as possible in order to detect clinical or serologic relapse. It is planned that all patients will again be hospitalized at the end of six months for complete restudy, which will include examination of their spinal fluid.

Should more extensive and prolonged experience confirm the impression which is to be gained from the pilot study, a rebuilding of the structure of syphilis therapy may be necessary. The development of an optimal therapy will require carefully controlled studies designed to determine the most effective relationship between the amount of drug and the duration of treatment. Also, the role of treatment for latent disease and for visceral and central nervous system syphilis will require careful study before the present methods of treatment can be replaced by penicillin therapy.

There were presented before the Section on Dermatology and Syphilology of the American Medical Association on June 15, 1944 three papers dealing with the use of penicillin for syphilis of human beings. The first of these, by Mahoney, Arnold, Sterner, Harris and Zwally,[64] expands the results reported in their original communication, of December 1943, to data on 100 or more patients with early syphilis treated with a standard dose of the drug, 1,200,000 units.

The second paper, by Moore, Mahoney, Schwartz, Sternberg and Wood[65] (the Penicillin Panel of the Subcommittee on Venereal Diseases, National Research Council), constitutes a preliminary report on 1,418 patients with early syphilis treated with penicillin. As a result of the original observations of Mahoney and his associates, there was organized on Sept. 1, 1943, under the general auspices of the Committee on Medical Research of the Office of Scientific Research and Development, and under the specific direction of the Subcommittee on Venereal Diseases, National Research Council, a cooperative study of the effect of penicillin on syphilis in human beings. Early syphilis is at present under investigation in 23 clinics or research centers, with the patients studied and treated in as nearly as possible a uniform manner and the results centralized and subjected to machine statistical analysis. In cases of early syphilis there appear to be five variables requiring study: first, the route of administration; second, the interval between injections; third, the duration of treatment; fourth, the total dosage, and fifth, the possibility of combination of penicillin with other drugs, e. g., mapharsen.

At the beginning of the cooperative study it was decided to hold the first three of these variables constant, i. e., all patients were to be treated by the intramuscular route every three hours day and night to a total of sixty injections given in seven and a half days. The first effort was to be to define the minimum effective dose so given within this period.

The preliminary results of the study, as summarized by Moore and his associates, are as follows: Penicillin has a profound immediate effect in early syphilis in terms of disappearance of surface organisms and open lesions, healing of lesions and a trend toward reversal of serologic reactions of the blood. These immediate effects are in general identical within a twenty-fold dosage range of 60,000 to 1,200,000 units administered by the intramuscular route every three hours day and night to a total of sixty injections within seven and a half days. The same immediate effects are apparent within the dosage range of 300,000 to 1,200,000 units given by the intramuscular route every three hours day and night to a total of thirty injections in four days. These immediate effects cannot be utilized to determine the optimum time-dose relationship, which in man depends on the incidence of relapse.

64. Mahoney, J. F.; Arnold, R. C.; Sterner, B. L.; Harris, A., and Zwally, M. R.: Penicillin Treatment of Early Syphilis, J. A. M. A. **126**:63 (Sept. 9) 1944.

65. Moore, J. E.; Mahoney, J. F.; Schwartz, W.; Sternberg, T., and Wood, W. B.: Treatment of Early Syphilis with Penicillin: Preliminary Report of 1,418 Cases, J. A. M. A. **126**:67 (Sept. 9) 1944.

The incidence of relapse in this series when penicillin has been administered alone, has been in direct relationship to the total dosage given by the intramuscular route in a seven and a half day period: greatest with 60,000 units and least with 1,200,000 units. Relapse appears to be more frequent after intravenous than after intramuscular administration of comparable doses. The lowest incidence of relapse and the most favorable serologic response were in a small group of patients treated with 300,000 units of penicillin plus a known subcurative dose of mapharsen.

Penicillin, it is reported by these workers, has a favorable effect in early asymptomatic neurosyphilis, acute syphilitic meningitis, early syphilis resistant to arsenic and bismuth, and infantile congenital syphilis. No opinion can as yet be expressed as to the effect of penicillin in the prevention of prenatal syphilis.

Herxheimer reactions after the penicillin treatment of early syphilis are frequent but not serious; other reactions due to penicillin itself are negligible.

Emphasizing the preliminary nature of their report, these workers say that the optimum time-dose relationship of penicillin in early syphilis is not yet established. Certainly the minimum dose, especially for secondary syphilis, should not be less than 1,200,000 units, probably it should be more.

Stokes,[66] reporting for the Penicillin Panel, provides preliminary results on the action of penicillin in late syphilis, including neurosyphilis, benign late syphilis and congenital syphilis. His group has studied 182 cases of late syphilis (including 122 of neurosyphilis and also including cases of benign gummatous syphilis, ocular and late forms of the acquired disease and late congenital syphilis) observed from eight to two hundred and fourteen days after penicillin therapy was begun on a wide range of time-dose schedules.

The following tentative observations are summarized:

(1) The lesions of benign gummatous syphilis of skin and bones heal under a dosage of approximately 300,000 units in 12 to 46 days.

(2) Irrespective of system used, and in all types of syphilis, penicillin causes reduction of syphilitic reagin titer in the blood in from 50 to 60 per cent of late cases. An initial "Herxheimer"-like or provocative rise is observed in about 20 per cent of cases. Only 5 seroresistant cases were treated, one made negative, four improved.

(3) The abnormal spinal fluid in neurosyphilis is improved in 74 per cent to some degree; markedly in 33 per cent. The commonest change is a drop in cell count and total protein (grade 2 improvement on a scale of 5) occurring in 67 per cent of cases. One spinal fluid was rendered normal within the observation period. All our fluid findings improved in 25 per cent of the cases of asymptomatic neurosyphilis, 10 per cent in paresis and taboparesis.

(4) Symptoms improved in neurosyphilis as follows: *Simple demented paresis:* In 30 cases on which data were adequate for classification, 80 per cent improved to some degree; nearly half improved 50 per cent or more, including eight who improved 75 per cent, and one restored to normal. *Deteriorated paresis:* 2 of 10 improved 75 per cent, one 50 per cent, 7 no change. *Tabes dorsalis:* one-fifth of 14 cases improved 50 per cent or more. Of 7 with lightning pains, 2 were completely relieved, 1 improved 50 per cent, 2 improved 25 per cent, 1 unchanged and 1 worse. Of 7 cases of primary (?) optic atrophy, mostly advanced, none was made worse, one improved. In meningovascular neurosyphilis 40 per cent improved 50 to 75 per cent.

(5) Two attempts at statistical evaluation were made. One, of the influences of smaller dose as contrasted with larger dose treatment; and the other, of the response under penicillin of spinal fluids with low as contrasted with relatively high cell counts, because of small numbers of cases and unavoidable disparities in observation period, cannot be accepted as beyond challenge. They suggest respectively that in late syphilis, especially neurosyphilis, smaller doses, if not grossly inadequate, have good effects which may perhaps be improved by repetition, as compared with the effects of initial larger doses—the effect being due perhaps to stimulation or utilization of the patient's resistance and defensive responses. The figures on response in relation to cell count suggest that moderate and high cell count cases tend to react somewhat better than cases with low cell counts.

(6) Previous treatment for syphilis by older methods in neurosyphilis, including fever therapy, does not appear to prepare patients for superior results with penicillin.

(7) *Late congenital syphilis*—interstitial keratitis presents rather equivocal though at times dramatically favorable results, not as yet interpretable in relation to time-dosage system. Of 14 cases, 6 improved, 3 to 100 per cent, 1 to 75 per cent, 2 to 50 per cent. Two were made definitely worse.

(8) *Optic neuritis* included 2 cases, both improved. The second 100 per cent on re-treatment. Iritis: 2 cases improved 100 per cent at the start, but one relapsed and did not respond to re-treatment (glaucoma).

(9) *Eighth nerve deafness,* 2 cases, equivocal results.

(10) *Miscellaneous cases,* Charcot joint unaffected (new one developing); gangrenous balanitis cured by low dosage.

(11) *Therapeutic shock (Herxheimer) effects.* These are undoubted, may be serious in late syphilis, and should be guarded against by reduced dosage during the first 24 to 48 hours. Severe cerebral and cord symptoms may develop in neurosyphilis.

Reactions to penicillin as such are few and not serious, urticaria, itching, allergic skin reactions, sharp gastrointestinal reaction following the course.

(To Be Concluded)

66. Stokes, J. H.; Sternberg, T. H.; Schwartz, W. H.; Mahoney, J. F.; Moore, J. E., and Wood, W. B., Jr.: The Action of Penicillin in Late Syphilis, J. A. M. A. **126**:73 (Sept. 9) 1944.

Book Reviews

Fundamentals of Internal Medicine. Second Edition. By Wallace M. Yater, M.D., Professor of Medicine, Georgetown University. Price, $10. Pp. XLI + 1204, with 275 illustrations and many tables. New York and London: D. Appleton-Century Company, Inc., 1944.

The author states that this book was designed to make available in simple form for students and practitioners the essentials of the entire subject of internal medicine. The first edition appeared in 1938 and was reprinted in 1940, 1941 and 1942; and the book now is in its second edition. For any book to have made such a record of popularity in so short a time means, of course, that it has had an unusually successful career.

The ARCHIVES reviewed the first edition on two occasions (**64**:1129 [Nov.] 1939; **67**:707-708 [March] 1941). Both reviews appear to have been written by practitioners rather than by students. Each reviewer confessed a slight disappointment at the character of the work, saying, in effect, that it was no more than an outline of medicine, so that adequate discussion of any important disease was lacking.

The second edition, which is the first brought up to date and revised, has been reviewed by a third year medical student. His impressions are interesting as perhaps revealing a more youthful point of view toward the book than has been hitherto expressed. This student believes that the book has filled a useful purpose: It offers any medical student a pleasant perspective of medicine, and it is well written, well illustrated and well printed. On the other hand, he suggests that the book is not altogether safe for the average student's use; its inherent aura of concentrated information in a small package may encourage intellectual laziness on the part of younger readers, and they may translate through the title more than the author has attempted to put into the pages.

No doubt, in spite of such possible shortcomings, the book will continue to have a wide circulation. On last analysis it makes an admirable adjunct to current texts on general medicine and if employed as the author intends it to be employed it will stimulate and help a great many physicians, both young and old.

A Textbook of Pathology: Pathologic Anatomy in Its Relation to the Causes, Pathogenesis and Clinical Manifestations of Disease. By Robert Allan Moore, M.D. Price, $10. Pp. 1338, with 513 illustrations. Philadelphia: W. B. Saunders Company, 1944.

This volume is an imposing affair, made up of 108 chapters grouped into seven major divisions. The manner in which pathology is thus dissected is the book's most novel feature.

As the author states, the broad division of pathology into general and special pathology is not controversial, nor is the further division of general pathology into disturbances of metabolism, inflammation and tumors. He believes, however, that the most desirable classification of disease is based on cause; therefore, in the chapters on special pathology diseases with similar causes have been grouped together. He has discussed diseases of obscure origin according to the organ or system in which they arise. Throughout the book he has attempted to emphasize the physiologic and chemical aspects of pathology rather than anatomic types.

The book is well indexed; the illustrations, some of which are in color, are satisfying, and at the end of each chapter is a carefully selected bibliography pertinent to the subject which has been under discussion. Most important of all, the author is a teacher who knows how to express his ideas clearly, logically and not too dogmatically.

Medical students will appreciate a textbook of this sort not only for the information it affords but because it is such an excellent tool for the encouragement of interesting collateral reading on any phase of medicine. As a model of a modern American textbook of pathology, it deserves a place in any library.

Sternal Puncture. Second Edition. By A. Piney, M.D., Physician, St. Mary's Hospital for Women and Children, London, and J. L. Hamilton-Paterson, M.D., Pathologist, Redhill County Hospital, Edgeware. Price, $3.50. Pp. xiv + 69, with 13 plates. New York: Grune and Stratton, 1944.

Sternal puncture is becoming increasingly popular in the United States. This small monograph coming from England, therefore, is timely and interesting.

At the outset the authors state that examination of marrow films is so new that it is still in the stage at which description is all that is possible. They then describe their observations in a variety of conditions, attempting to prove that a myelogram well made and interpreted wisely may yield valuable information.

The first chapter describes the myelogram and the last the technic of sternal puncture; sandwiched between are eight chapters which describe what marrow films show in the leukemias, the anemias, infections, certain protozoal diseases and erythremia.

One of the most pleasing features of the book is its illustrations. All but one are colored; each is clearly labeled; they are so attractive to look at as to make the casual observer anxious to become a hematologist and familiar with the precursors and variants of those blood cells which he sees every day when he looks at a blood smear.

Lord Horder wrote a foreword to the first edition. He said that the popularity achieved by Dr. Piney's previous books in hematology might safely be predicted for this new work. That a second edition of it was needed in England within two years is good evidence of Lord Horder's shrewdness. The book should please physicians and students on this side of the Atlantic too.